

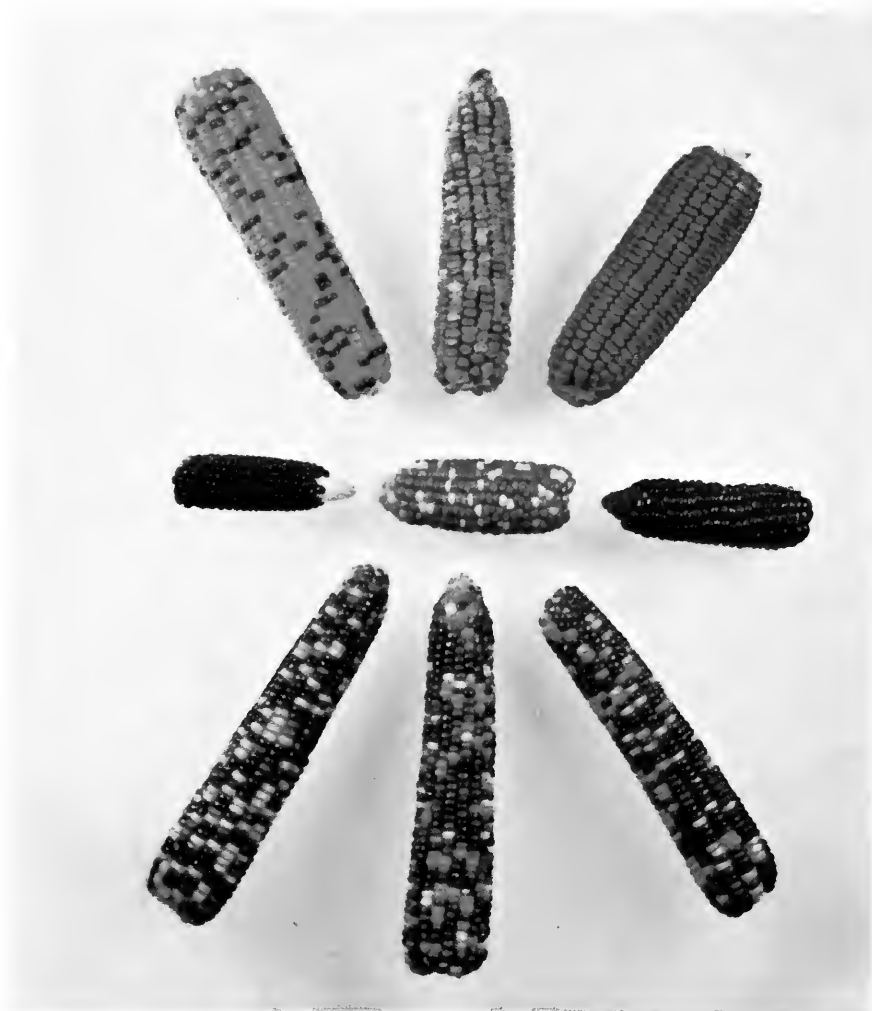


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Segregation in corn.

Elementary

GENETICS

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This text is dedicated to the memory of

WILLIAM ERNEST CASTLE

and

EDWARD MURRAY EAST

of the Bussey Institution of Harvard University
Teachers and leaders in the
formative years of Genetics

Preface

Elementary Genetics is designed as a comprehensive introduction to the subject. The science of genetics has expanded so rapidly in its six decades of existence that there is a wealth of material to draw on. For the sake of simple and direct presentation of the basic principles, examples have been carefully chosen. Some are traditional—*Drosophila* and maize. *Neurospora* also appears from Chapter 1 forward throughout the text. There is no special chapter on genetics of microorganisms, any more than on maize or *Drosophila*, but microorganisms are discussed in many places in the text, wherever they are useful to illustrate a genetic principle. In presenting some newer ways of transferring genetic material, such as transduction and transformation, the microorganisms are the only illustrative material.

Hereditary examples are drawn from a wide range of living things, from phage to *Pferde* (horses). There is a comprehensive chapter on coat color inheritance in mammals. A chapter is devoted to the comparatively new field of biochemical genetics, and another to biochemical genetics in man.

Some traditional illustrations of heredity will not be found in this book. For instance, I have not brought in the inheritance of blue versus brown eyes in human beings. There are so many genes modifying eye color that it is difficult, without a breeding test, to tell the true inheritance or genotype of an individual. Much unhappiness has been caused by a literal interpretation of the dictum that the gene for blue eyes is recessive and that blue-eyed parents can never have a brown-eyed child. Eyes that are blue-gray may in fact be the manifestation of a heterozygote for blue and brown. Two such persons could very well have a brown-eyed child. Modifiers undoubtedly affect the major gene for eye color, and it is highly probable that there are cases of low expressivity of the gene for brown that has produced apparently blue eyes in the parent but could give rise to brown eyes in the offspring. For this reason the inheritance of eye color in human beings will not be dealt with in the text. This is the last reference to it.

Some examples in the book are also new—for instance, the character so much more striking and also so much more constant than eye color, red hair in human beings. Why this has been neglected for so long as an example of a hereditary character is something of a mystery. The gene for red behaves

like a simple Mendelian recessive to non-red, which can either be blond or brunette. There are genes that modify the shade of red hair, but the difference between red and non-red is always clear-cut. Since completing this text, I have seen an extensive pedigree chart of a family for six generations, in which the gene for red was segregating. This was prepared from her own family records by Miss Brenda Ellis, of Carson-Newman College in Tennessee. All pedigrees are consistent with the interpretation that the gene for red is a monogenic recessive to non-red, in complete agreement with the analysis presented in this text. I am grateful to Miss Ellis for permission to cite this pedigree chart.

The glossary should prove helpful to a student encountering genetics for the first time. It translates some of the scientific jargon into English.

Of the more than two hundred illustrations, a large number represent photographs (several from Brookhaven National Laboratory). More than fifty drawings are the work of Te-Hsiu Ma, my first graduate student, who is both a professional geneticist and an artist. We want to thank all those who supplied us with illustrations, and in particular to make mention of Miss Charlotte Auerbach, and her publisher, Oliver and Boyd, Ltd., as well as the American publisher (Essential Books, Inc.) who granted us permission to reproduce several illustrations from *Genetics in the Atomic Age*. We have also adopted her style of two-color drawings in one of our drawings showing the inheritance of red hair in man.

Dr. C. C. Little and the Comstock Publishing Associates granted us permission to reproduce several drawings and dog pedigrees from *The Inheritance of Coat Color in Dogs*.

We have used several of the excellent illustrations from *Cell Heredity* by Ruth Sager and Francis S. Ryan with their permission and that of John Wiley and Sons, the publisher. We are most appreciative of these.

We wish to thank H. E. Warmke for permission to reproduce several excellent drawings first published in the American Journal of Botany of the chromosomes of *Melandrium*, showing the sex chromosomes.

The distribution of the chi-square values for Chapter 5 is abridged from a table in *Statistical Tables for Biological, Agricultural and Medical Research*, published by Oliver and Boyd, Ltd. We wish to thank Sir Ronald A. Fisher and Frank Yates for permission to abridge and reproduce this table.

We are most grateful to Loring Jones and the De Kalb Agricultural Association, Inc. for permission to use the plates of their booklet *The Ten Chromosomes of Maize* as Appendix A. Margaret Green and Margaret Dickie kindly permitted us to reproduce a linkage map of the 20 mouse chromosomes, as well as a description of the genetic characters of the mouse, first published in the *Journal of Heredity*, as Appendix B.

The writing of a textbook represents the efforts of a number of people. In a way the author is sometimes more of an editor than an author. Illus-

trative material must be selected with care and permission obtained for its use. My friends and colleagues were most kind and generous, granting permission whenever requested. The editorial staff of the D. Van Nostrand Company showed patience with the author when it was needed, and also a little tactful prodding when this was in place. A special word of appreciation is due the publisher's reader of the manuscript. Friends read portions of the manuscript and made valuable suggestions. Addison Lee of the University of Texas and Gertrude Heidenthal of Russell Sage College were particularly helpful. My secretary, Mrs. Anna Kirby, typed the whole manuscript with remarkable care. And finally I am most grateful for the assistance of my wife, Dorothy Amrine Singleton, in reading the whole manuscript, and suggesting improvements in many places. She also did the preliminary copy editing for the publisher and helped shepherd the manuscript through galley and page proofs.

After this book was printed news came of the death of Dr. W. E. Castle on 3 June in his 95th year. I am indebted to him in many ways, first as one of his students, later as a collaborator in genetic studies in horses. He read and improved Chapter 10 on the inheritance of coat color in mammals. Several of the illustrations in Chapter 10 were from his early work published in the *Journal of Heredity*. He maintained his research interest to the end, having written four research papers in the last two years. Geneticists have lost an eminent teacher, leader, and friend.

Although many people assisted in the preparation and production of this text, the author alone is responsible for any errors that may appear. It is the author's firm belief that any text should be revised within three to five years after publication. In a subject where new developments come as rapidly as they do in genetics, it is imperative that textbooks be frequently revised, and the author would appreciate help from readers in preparation for the next revision of his.

Charlottesville, Virginia
6 June, 1962

W. R. S.

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Genetics: The Science of Heredity

WEBSTER DEFINES genetics as “the branch of biology which deals with heredity and variation among related organisms, largely in their evolutionary aspects.” It is a study of differences in living things. The life blood of the science of genetics is the variation that occurs within and among plant and animal species. Although there could be other scientific biological studies—such as physiology, nutrition, and morphology—of organisms essentially alike, there could be no science of genetics without variation.

A difference, however, is not enough for the establishment of the laws of heredity. Fig. 1-1 shows what a tremendous difference in size there can be between animals of the same species. The weight ratio is approximately 100:1 for adults of the same species, perhaps the greatest difference in any animal species. Presumably an artificial mating could be made between the St. Bernard and the Chihuahua and fertile progeny obtained. It is doubtful, however, if any exact laws of heredity could be worked out in a reasonable time by studying such a wide cross. On the contrary, the basic principles of genetics have been developed by studying smaller differences, those affecting only one or at the most a few visible characters.

SEVEN HUNDRED ALBINO SQUIRRELS

A good example of a mutation is the albino squirrel (Fig. 1-2). Here is a striking difference that occurred in a population of gray squirrels. About 1905 two white squirrels, presumably albino, were liberated in Olney, Illinois, and from these developed a colony of approximately 700 squirrels. These squirrels have not been subjected to genetic tests of crossing with the common gray squirrel, but there is little doubt that they have a definite hereditary character similar to the albino mice, rats, and rabbits. This albino type

must have arisen by mutation in the wild gray squirrel. More will be said about mutation in later chapters. It is the source of all variation in living things, the very building substance of evolution. All of the variants that we study in genetics today have arisen by mutation from a wild type.

The occurrence of albinos in rodents is familiar to all. Such animals are pure white, possessing no melanin pigment. The eyes appear pink simply be-



Courtesy of Gaines Dog Research Center, New York City

FIG. 1-1 Extreme size difference in single species—the adult St. Bernard is approximately 100 times as heavy as the Chihuahua.

cause there is no pigment in the iris, so that the blood vessels in the back of the eye are visible, giving the eye a definite pink cast. Albinos have been observed in deer, and also in at least one bird, the robin. Albinos also occur among human beings. In fact, the characteristic is seen in a wide variety of organisms, and its biochemistry is now fairly well known (discussed in Chapter 19).

Albinos are at a distinct disadvantage in nature, since they are easy prey and thus are usually eliminated quickly from the population. The only in-

stance known to the author of a large population built up in a more or less wild state is the Olney, Illinois, case. The squirrels are protected by a city ordinance passed in 1925. The white squirrels have the right of way on any street in the town, and there is a fine of \$25 for killing one. An additional case of the albino squirrel was noted in Richmond, Virginia, in the fall of 1959 in a news story in the *Richmond News Leader*, undoubtedly a different mutation



Courtesy of Harry Hillis, Jr., Olney; and William Bey, Lawrenceville, Illinois

FIG. 1-2 Ten of the members of a colony of albino squirrels in Olney, Illinois.

from the one observed in Illinois. The albino mutation has also occurred in gray squirrels near Berryville, Virginia.

A contrasting mutant type of squirrel observed in the wild is completely black. Such an animal was seen a few years ago in one of the many parks in Washington, D. C. It is possible that in time a colony of black squirrels might be established in the same way as the white colony in Illinois.

RECESSIVE RED HAIR IN HUMAN BEINGS

We need not limit ourselves to wild animals in looking for striking differences among individuals. The case of albinism in human beings was cited earlier. Another striking character in human beings is red hair, examples of which are easily observed every day. Most red hair has a genetic basis, but hair dyeing has become so popular that one should be a little cautious in deciding that a redhead is genetic and not synthetic. If one knows the parents and observes the offspring as children, one can be certain the red is genetic.

An old adage regarding heredity is that "like produces like." In some cases this is true. In a community where the author once lived was a family of four redheaded girls (Plate I). The father had red hair (or what little was left of it), and the mother's hair was also red, although tinged with gray. So here was a case of like producing like. This is perfectly sound genetics, since red hair is a *recessive* trait.

Since this is our first encounter with the term *recessive*, a brief definition is in order. In some marriages, for example between persons of red and brown hair, all the children are brown-haired, provided the parents represent pure strains of this gene. In other words, the red seems to recede completely in the offspring, while the brown seems to dominate. For this reason the red is termed *recessive*, the brown *dominant*. These terms were coined by Gregor Mendel, the father of genetics, a century or so ago.

Some definitions of other technical terms are necessary. We have already used the term *gene*, which may be described as the hereditary determiner, whatever it is. In recent years much has been learned about the nature of the gene, which will be discussed in Chapter 22. For the present it is sufficient to know that the gene is the hereditary determiner. Mendel called these determiners "elements." Some geneticists have called them "factors." We term them "genes." Elements, factors, or genes, they transmit individual traits called "characters." Since Mendel was the first to suggest a particulate determiner of heredity, it can be truly said of him that he discovered the gene. The name was given much later by a distinguished Danish geneticist, W. Johannsen.

Genes come in pairs of forms, one member of each pair having arisen by mutation from the other. These alternative forms of the gene, once called «*allelomorphs*» [Gr. *allēlōn* of one another + *morphē* form] are now called *alleles*. They may be described as either of a pair of alternative determiners for contrasting characters such as red and black hair in man. One member of each pair of alleles is the normal, or wild-type gene, the other member the mutant gene.

Every individual among higher plants and animals begins life as a fertilized egg. The father contributes the sperm, the mother the egg, each with a full complement of *chromosomes*, which are rodlike bodies containing the genes. The chromosomes derive their name from the fact that, when observed in stained material, they are deeply colored, i.e., "color bodies," or chromosomes. The genes are located in the chromosome. In human beings each individual receives 23 chromosomes from the mother and 23 from the father. The egg and sperm are known as *gametes* [Gr. *gametē* wife, or *gametēs* husband]. The union of two gametes produces a *zygote* [Gr. *zygōtos* yoked] with double the chromosome number (diploid) of that possessed by the gamete (haploid).

Let us return to the family of four red-haired girls, each of which must have been *homozygous* [Gr. *homo-* like, *zygon* yoke] for the gene for red hair. There can be different shades of red hair, and in this family there were. The first two daughters had flaming red hair, while the third and fourth girls had

hair that tended to be a reddish gold. The hair of all four was clearly recognizable as red (Plate I). Actually there may be modifying genes that act on the gene for red hair, and it is conceivable that more than two alleles of this gene may exist. In fact, it seems quite likely that the auburn shade of hair is conditioned by a different allele or perhaps by another gene. This is somewhat analogous to coat color in dogs, where the red of the Irish Setter is due to one gene while the golden type of the Labrador Retriever is conditioned by the interaction of at least two genes. Also, the chocolate brown coat of the Chesapeake Bay Retriever is determined by a gene that changes the black pigment to a chocolate brown color. It is possible that the auburn hair in human beings might be conditioned in a manner similar to this.

We know that there can be various shades of red hair among human beings, and it is not possible to state whether more than one gene pair is involved. It is possible that there is one major gene pair with several modifying genes.

Many readers may have read or heard of *Life with Father*, which depicts the life of Clarence Day and his family. In this family there were four redheaded sons. Of course the mother and father had red hair—perfectly good genetics. Again, like produced like.

However, the alternative of this occurrence is just as sound. I knew a family at the Brookhaven National Laboratory in which the father and mother both had black hair and the first child, a daughter, had black hair. Like produced like again. But the second child, a son, had brilliant red hair. Here like did not produce like. However, this child gave us information about the genetic constitution (the *genotypes*) of the parents. It showed that the mother and father were both *heterozygous* [Gr. *hetero*- other, *zygon* yoke] for the gene for red hair. In other words, they were not pure for black hair. Each parent must have received the allele for red from one parent, while receiving the alternative of this, the allele for “non-red,” from the other parent. Their physical appearance (*phenotype*) was dark, but their genotype must have consisted of one allele for red while the other allele was for non-red.

SHORTHAND DESIGNATION OF GENOTYPE AND PHENOTYPE—GENE SYMBOLS

It is a common custom among geneticists to use italic letters as symbols for genes. A capital letter indicates a dominant allele, the corresponding small letter the recessive. The particular letter chosen indicates the mutant gene. For example, in the case of red hair in human beings we can let *r* stand for the red mutant allele, while *R* would designate the allele for non-red hair. The *R* could stand for black hair, or even blond, different manifestations of the non-red allele of the gene pair concerned with hair color.

In some of the animal genetics the letter *e* is used to indicate red (*e* standing for the Greek word *erythros*, meaning red). The letters *e* and *E* might just as well be used to designate red hair and non-red hair, respectively. Mendel

simply wrote the letters A and a to indicate dominant and recessive. Any terms are purely arbitrary and the conventional ones of most geneticists today will be used here. If the R/r terminology is used, it is obvious that the genotype of both of the parents mentioned is R/r , since each is heterozygous. Since they are both of the genotype R/r , they can produce two kinds of gametes, either R or r . The union of two r gametes would produce a redheaded individual, as actually happened in the case of the second child of the family at the Brookhaven National Laboratory in Long Island, New York.

The homozygous recessive is written r/r , the homozygous dominant R/R , and the heterozygous individual R/r . Since R is dominant, R/R and R/r individuals are indistinguishable phenotypically. It is desirable to have a shorthand designation for persons who are phenotypically dark, but whose actual genotype could be R/R or R/r . Such individuals are designated $R/—$. Before the birth of the second child of the Brookhaven family, the genotype of both parents had to be written $R/—$. After the birth of the second child (r/r), the genotype of the parents became known and could be written R/r .

Since the R and r alleles would each be produced in 50% of the cases, the probability of either being produced is one half. The chance that two gametes with r alleles would combine to form a zygote is the product of the individual probabilities of producing an r allele, or $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$. This gives us the familiar one-fourth of a population that is expected to be r/r when two heterozygous R/r individuals mate. Such 3:1 Mendelian ratios will be discussed more thoroughly in Chapter 3.

In a Virginia family, the father and mother both have dark hair and the first child has brilliant red hair (Plate I). The other two children have hair color that is brown—somewhat lighter than the parents, but definitely not red. Here we have a ratio of two non-red (R) to one red (r/r), which appears nearer the expected 3:1 ratio than the 1:1 ratio of the Long Island family cited earlier. Both of these numbers are close approximations of a 3:1 ratio, as near as we can expect with small numbers. In Chapter 5 deviations from expected ratios will be discussed in a test for significance of such deviations.

A third family, in which both husband and wife are heterozygous for red (R/r), lives in Charlottesville, Virginia. They have two children, both with red hair. The probability for such an occurrence is $\frac{1}{4} \times \frac{1}{4}$ or $\frac{1}{16}$. In the case of this family, each parent with unusually dark hair, had one parent with red hair, thus proving that each is heterozygous, as the progeny demonstrated. Still another family in Charlottesville demonstrates that both mother and father are heterozygous (R/r). Both have extremely dark hair. The first two children have brown hair while the third child has brilliant red hair. In the mother's family, red hair was common, but none was known in the immediate ancestors of the father. This demonstrates the fact that a recessive gene may be carried for several generations in the heterozygous condition. It is only when the recessive red (r) gamete meets another r gamete that a red zygote results, definitely proving the heterozygous (R/r) nature of the parents.

These examples of inheritance of hair color are given because of their striking nature. They are quite familiar. Their laws of heredity are now well established. What is not so certain is *how* these changes in hair color are brought about. Actually there are hereditary determiners for red or dark hair carried in the chromosomes. Somewhere among the 23 pairs of chromosomes in the human species is one pair carrying two alleles for red (r/r), two alleles for non-red (R/R) or one allele for red and one for non-red (R/r). These always occur in pairs and each of the gametes gets either one or the other, not both. If a zygote gets two R alleles, it is pure or homozygous for the brown hair color (R/R). Such an individual will breed true for dark hair. A person having an R allele along with an r allele is heterozygous R/r and dark haired, but will not breed true when mated with an R/r or r/r individual. One with the constitution r/r has red hair and will breed true for red, that is, if mated with another r/r individual.

ABILITY TO TASTE PHENYL-THIO-CARBAMIDE (PTC)

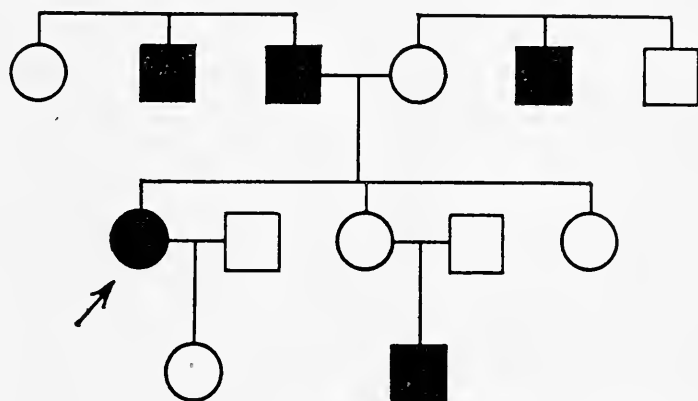
Another easily discernible hereditary character in man is the ability or inability to taste a substance such as phenyl-thio-carbamide, or PTC. To some people this has a distinctly bitter taste, while to others it is tasteless. One of the 23 pairs of chromosomes carries the genetic specification that a person can or cannot taste this particular substance. Since tasting is dominant we label it T , while the non-tasting is designated t . It seems most likely that tasting (T) is the normal condition and non-tasting (t) represents a mutation from T . As far as is known, these alleles do not have any influence on the ability to taste other substances. A person to whom the substance tastes bitter has the genetic constitution of either T/T or T/t , and it is not possible to tell except by testing the progeny whether the constitution is T/T or T/t . In the author's family, he is a taster and must be either T/T or T/t . His wife is a non-taster, t/t . By testing the children it is possible to tell whether the father's genotype is T/T or T/t . Actually three of his four children are tasters ($T/\text{—}$), whereas one is a definite non-taster (t/t). Thus his genotype is T/t and not T/T . If it had been T/T all of the children would have been tasters regardless of the genotype of the mother.

Since the mother is a non-taster (t/t), it was interesting to run the taste test on her parents. Actually both parents were tasters, indicating they were both heterozygous (T/t). If either had been T/T they could not have had a t/t daughter. The foregoing illustrations show what types of information can be secured by a knowledge of genetics. This is one of the things that makes genetics an interesting and personal science.

INHERITANCE OF SEX-LINKED CHARACTERS

A striking hereditary character in man is red-green color blindness. This is conditioned by a recessive gene located in the X chromosome.

The X and Y chromosomes are the ones concerned with the determination of sex. A female has two X chromosomes (X/X) and is called the *homogametic sex* because she can produce but one kind of gamete, i.e., with an X chromosome. The male is X/Y and is called the *heterogametic sex* because he can produce two kinds of sperm, or gametes, with either an X or a Y chromosome. Color blindness is more frequent in males, as they do not have another X chromosome which could carry a dominant normal gene to mask the expression of the recessive gene for color blindness in the other. Hemophilia is also caused by a gene located in the X chromosome (h). Consequently many more male children are subject to this disease than are female children. It can occur in females as can color blindness, but in such individuals both of the X chromosomes must carry the recessive condition (h/h). Until recently it was



Courtesy of Curt Stern; and the Freeman Company

FIG. 1-3 Pedigree of hemophilia containing a hemophilic woman. Circles represent females, squares males. Affected persons are shown in solid circles and squares.

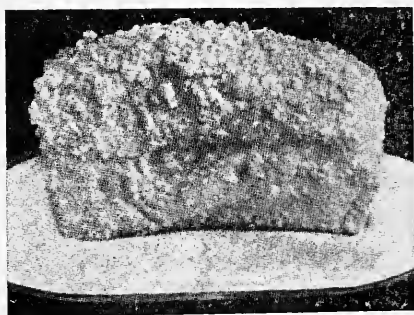
thought that the homozygous condition was lethal. A case has been described in 1951 (Israel, *et al.*) of a hemophilic woman (h/h) (Fig. 1-3). The inheritance of genes located in the X chromosome will be covered thoroughly in Chapter 7.

Not only in human beings, but in many other living things as well, we see evidence of genetic differences. Coat color in pets is easy to observe. As a geneticist, if you should see a calico or tortoise-shell cat, one with black, yellow and white markings, even across the street, you would know this is a female cat. The reason you can be sure is that the tortoise-shell cat is a hybrid between black and yellow, and this gene pair is located in the X chromosome. You will learn all about this in Chapter 7, which will explain why the males have only *one* X or sex chromosome and cannot be heterozygous for any character located in the X chromosome. Thus a male cat can be yellow or black but *not* a combination of the two, such as tortoise-shell.

INHERITANCE IN LOWER FORMS OF LIFE

The foregoing examples of genetic differences have been taken from the animal kingdom. However, the same laws of inheritance apply to plants as well, even to such lower forms as bacteria. Also, many genetic characters are known in viruses such as bacteriophage, the simplest form of living matter.

A notable example of a genetic difference in one of the lower forms of plant life lies in the colors of the common bread mold, or *Neurospora*. The genetics of this was developed in the late 1920's by B. O. Dodge in a careful scientific analysis. Although the individual threads of the mycelium of a bread mold are too narrow to be resolved by the human eye, the tangled mass of threads (the mycelia) is very conspicuous and may cover completely



Courtesy of the late B. O. Dodge; and Mycologia

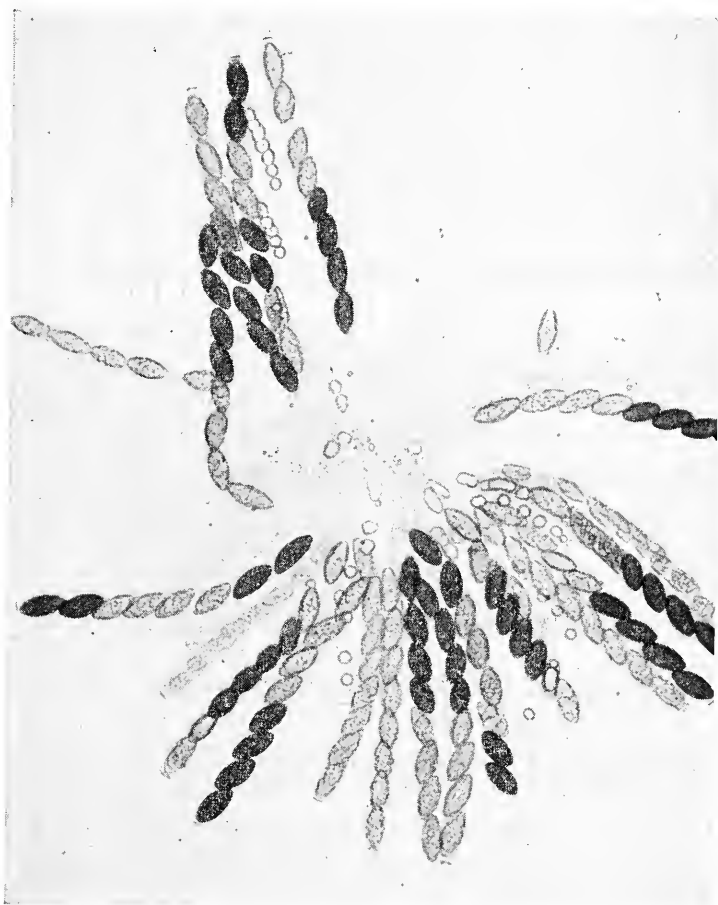
FIG. 1-4 Albinistic (left) and normal red type (right) in *Neurospora*.

a loaf of bread. A good example of phenotypically different mycelia is seen in Fig. 1-4, in which the red strain photographs as dark, in contrast to the white or light strain. A hybrid of *Neurospora* segregating for light and dark ascospores is shown in Fig. 1-5. Note that there are equal numbers of the dark and light spores. Because one can determine the genetic type of all eight ascospores, *Neurospora* is an excellent genetic tool. More about this will appear in Chapter 18.

INHERITANCE IN MAIZE

Many genetic variations occur in maize (corn), which has been analyzed more completely from a genetic standpoint than any other plant (see Appendix A for a description of genetic characters). Two examples of rather large differences are used as illustrations. Fig. 1-6 shows extremes for plant height. The shorter type produces a greater ratio of grain to stalk and may have commercial possibilities. The second illustration of an extreme

difference caused by a single dominant gene is corn grass (Fig. 1-7). This arose as a spontaneous mutant in a farmer's field. Its inheritance has been determined. Corn grass is due to a single dominant gene located in chromosome 3. This one gene can make a change from an erect, single-stalked plant



Courtesy of David R. Stadler

FIG. 1-5 Segregation of light and dark ascospores in *Neurospora*, a monogenic difference.

to a rather low-growing, grasslike plant with many stalks. It is conceivable that the primitive ancestor of corn could have been like this in appearance. It could have grown for centuries before it mutated to a type with an erect stalk resembling the present-day corn. Such a plant could then have been selected and developed into the corn plant that was growing here when Columbus discovered America.

INHERITANCE IN DOMESTICATED ANIMALS

An explanation of the inheritance of a few other characters in domesticated animals will be given. When I was a small boy on a farm in eastern Washington, I was intrigued with the Blue Andalusian fowl. Each spring I wanted to get some eggs of this breed for hatching, but I was always told by my father that this was not a good breed—it would not breed true.



Courtesy of Brookhaven National Laboratory

FIG. 1-6 Short corn determined by a single recessive gene.

Later, in studying genetics, I learned that the Blue Andalusian is a cross between a black and a white.

The expression of the hybrid is blue or slate gray in appearance. If we let B/B represent black and b/b white, we can see that the hybrid would be B/b . This is the blue condition that forms both B and b gametes. From such a hybrid it is possible to recover B/B and b/b , both of which will breed true, but the B/b hybrid condition can never breed true. Here is a case where neither allele is dominant over the other, but each allele in the heterozygous condition B/b produces about half the effect of the two alleles when homozygous, either B/B or b/b . Although such cases are less numerous than those showing dominance of one allele, they do occur. In this instance the black parent was designated with B/B and the white parent b/b , indicating domi-

nance of the black according to previous use of capital and small letters. In cases where the hybrid is intermediate between the parents, the choice of a capital letter for one allele and a small letter for the other is purely arbitrary. Neither is dominant or recessive.



Courtesy of Brookhaven National Laboratory

FIG. 1-7 "Corn grass" and normal corn plants. A single dominant gene is responsible for grassy plants.

The Palomino breed of horses is also hybrid. Like the Blue Andalusian fowl, it will not breed true. The palomino color is due to a dilution gene which in the heterozygous condition dilutes the normal reddish color of a chestnut animal to a beautiful golden color with a lighter mane and tail. If the dilution gene is homozygous (D/D), the second D allele causes further reduction in pigment, producing an animal that is almost white, a cremello, not nearly as desirable a color as the palomino.

Palomino breeders have been satisfied to breed their animals knowing that they will get only about 50 per cent of the desired color; the other 50 per cent will be divided equally. William E. Castle, dean of mammalian geneticists, has devised a method for securing 100 per cent palominos by mating a

cremello with a chestnut, neither parent of which is palomino. The method is feasible and is being used by horse and pony breeders to produce palominos (Castle and Singleton, 1961).

One fourth of the progeny resulting from mating palominos will be cremello with two dilution genes, and one fourth chestnut, with no dilution genes according to the formula $D/d \times D/d = \frac{1}{4} D/D$ (cremello), $\frac{1}{2} D/d$ (palomino), and $\frac{1}{4} d/d$ (chestnut) Plate II.

CONCLUDING REMARKS

Genetics is concerned with a study of inheritance of differences. Without such variations in living organisms, there could be no science of genetics. All laws of heredity have been developed by studies of differences conditioned by a single gene. Many mutants, mostly recessive, have arisen by mutation from the wild type.

Examples of mutants determined by a single recessive gene in the homozygous condition are: albinism in animals including man (c/c); red hair in human beings (r/r); the inability to taste PTC (t/t); and short corn (rd/rd).

A dominant mutation in corn is known as corn grass ($Cg/-$). In some mutations there is no dominance, as in the palomino color of horses. The inheritance of the palomino color was discussed.

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PROBLEMS

1-1. Discuss the following terms:

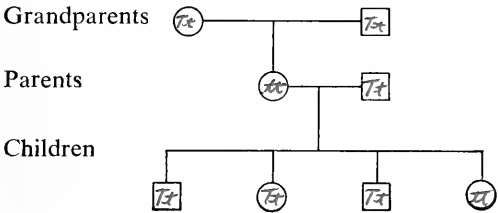
albino	heterogametic sex
allele	heterozygous
Blue Andalusian	homogametic sex
calico cat	homozygous
chromosome	maize
color blindness	mutation
corn grass	Neurospora
cremello	Palomino
diploid	PTC (phenyl-thio-carbamide)
dominant	phenotype
gamete	recessive
gene	sex-linked
genotype	tortoise-shell cat
haploid	zygote
hemophilia	

1-2. Identify the following scientists, giving a major contribution of each, with an approximate date.

Castle, W. E.	Johannsen, W.
Dodge, B. O.	Mendel, Gregor

- 1-3. Suppose a redheaded woman marries a dark-haired man (non-red). They have two children, both with red hair. Could you say whether red is recessive or dominant? (Until recently, it was uncertain which was dominant.)
- 1-4. In the marriage of two redheaded persons, there were four daughters, all with red hair. Would this example help in solving the problem of dominance?
- 1-5. In another marriage of a redheaded woman and a dark-haired man, there were three daughters, all with dark hair. Write a possible genotype for the father and the mother, letting a capital letter *R* stand for the dominant condition with the small letter *r* indicating the recessive.
- 1-6. In the marriage of two persons with dark hair, there were three children, two with dark hair and one with brilliant red. Write the genotypes of the father, mother, and children. Does this example show conclusively which color is dominant?
- 1-7. The gene for dilution of coat color in horses is commonly labeled *D*. An animal homozygous for *d/d* has the red coat characteristic of chestnut or sorrel horses. A *D/d* horse is a beautiful golden color with a white mane and tail. A homozygous *D/D* horse is known as a cremello. It has a definite

- cream color (hence the name), and blue eyes. Which is dominant—dilution or no dilution?
- 1-8. The Blue Andalusian breed of chicken, slate gray in color, is a hybrid between a black chicken and a white one with inconspicuous flecks of black. Which color is dominant? Write possible genotypes of the parents and of the hybrid.
- 1-9. A calico cat mates with a yellow tomcat. Designate yellow— Y , black— B . Write the genotype of the calico female and of the yellow male. (Remember that a male cat has but one X chromosome.) How many kinds of gametes will be produced by the female and by the male? In a litter of four kittens, two males and two females, if one assumes that all possible types of gametes are produced by males and females, what will be the colors of the male and female kittens?
- 1-10. Two persons who are tasters for PTC have a daughter who is a non-taster. Taster is dominant (T) over non-taster (t). The daughter marries a man who is a taster. They have four children, three tasters and one girl who is a non-taster. Fill in the pedigree chart of grandparents, parents, and children. Circles represent females, squares males.



Physical Basis of Heredity

THE GENES that initiate the action leading to distinct hereditary characters are located in the chromosomes. The basic laws of heredity were determined before there was any knowledge regarding chromosomes, which we now know are the carriers of the genes. It is truly remarkable that Mendel developed such precise laws of heredity without knowing about the locations of the genes (elements) and about the intricate process of cell division that produces the gametes. His reasoning, however, was sound.

. . . the conclusion appears logical that in the ovaries of the hybrids there are formed as many sorts of egg cells, and in the anthers as many sorts of pollen cells, as there are possible combination forms, and that these egg and pollen cells agree in their internal composition with those of the separate forms.

In point of fact, it is possible to demonstrate theoretically that this hypothesis would fully suffice to account for the development of the hybrids in the separate generations, if we might at the same time assume that the various kinds of egg and pollen cells were formed in the hybrids on the average in equal numbers.*

Although Mendel knew nothing of chromosomes, the carriers of the genes, he developed a correct theory as to how the determiners of heredity are distributed in the production of germ cells.

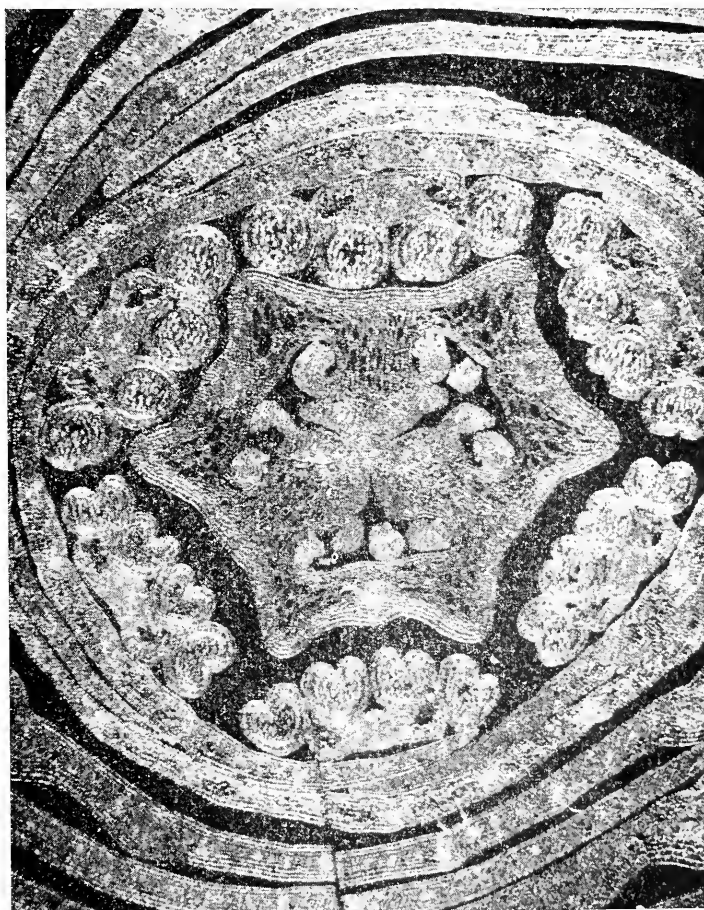
Between the time that Mendel presented his results in 1865 and the time his paper was rediscovered in 1900 by Correns, De Vries and Tschermak, there was much activity in studying the structure of cells of plants and animals. In 1875 Strasburger discovered the fact that, when a cell divides, the nucleus becomes separated into rodlike bodies called chromosomes. Many workers contributed to the knowledge of the physical chromosomes and of the hypo-

* All excerpts from English translation of Gregor Mendel's "Experiments in Plant Hybridisation" are reproduced by permission of the Royal Horticultural Society, London.

thetical determiner of heredity, the “element” of Mendel, or the gene of present-day usage. A summary of the interesting history of genetics can be found in *Great Experiments in Biology* by Gabriel and Fogel (1955).

The first general treatise that correlated the regularities of the distribution of the genes with the physical chromosome mechanics was made by Walter S. Sutton in 1902. His paper, “The Chromosomes in Heredity,” can be found in *Great Experiments in Biology*, just cited.

All plants and animals are composed of cells, either singly in lower forms such as bacteria and protozoa, or in rather complex organizational patterns as found in higher plants and animals. Each cell has a nucleus, rich in a highly complex *nucleoprotein* called deoxyribonucleic acid, abbreviated for convenience to DNA. Much has been learned about DNA in recent years.

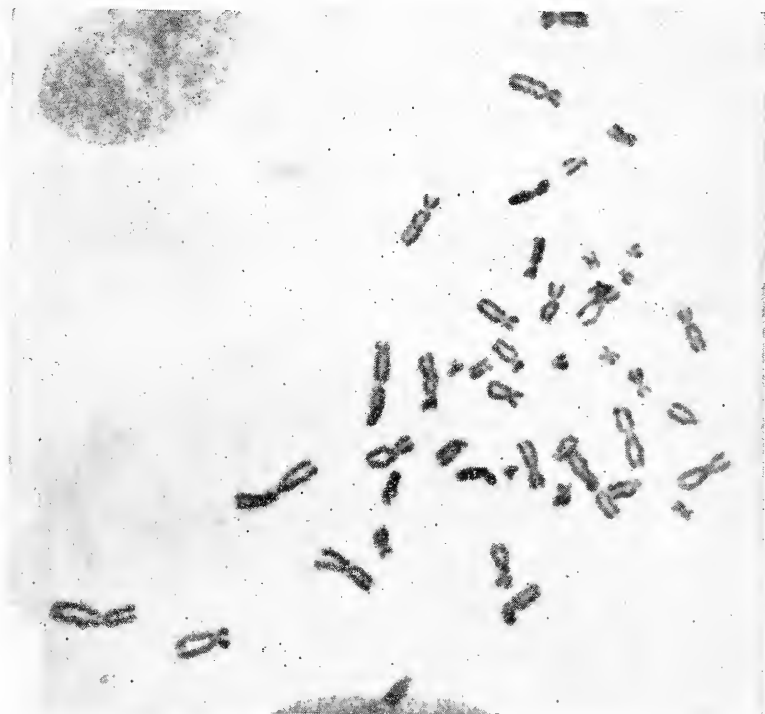


Courtesy of A. H. Sparrow; and Brookhaven National Laboratory

FIG. 2-1 Cross section through a bud of *Trillium* (wake robin). Ovary in the center is surrounded by six anthers of four locules each.

It will be discussed more fully in Chapter 22. It appears to be the basic living substance, capable of duplicating itself to form more DNA, a prerequisite of any living thing.

Fig. 2-1 shows a rather complex organizational pattern of a flower bud in the common wake robin, or Trillium, a monocotyledon in the family Liliaceae. Note the star-shaped ovary in the center surrounded by the six anthers of four parts or locules each. This Trillium plant began life as a single cell, the product



Courtesy of H. Y. Chu, Oak Ridge National Laboratory

2-2 Human chromosomes in somatic tissue. At meiosis the 46 chromosomes form 23 pairs.

ertilization of the egg in the ovary, by a sperm nucleus from the pollen is fertilized egg, or zygote, has divided many times until we have the pattern seen in Fig. 2-1. The process by which an undifferentiated fertilized egg has arrived at such a complex organization is not completely understood, but much is known about the mechanics of cell division, especially of the nucleus.

Shortly before a cell divides, the nucleus undergoes radical changes. The nucleus is made up of a transparent mass of protoplasm, known as *karyolymph*, which is surrounded by a thin nuclear membrane. In the karyolymph

are embedded long, very narrow threads, the chromosomes. These threads never break up into unorganized material, as was long supposed, but maintain their integrity of organization from cell division to cell division. As preparation for nuclear reproduction, the chromosomes shorten up until they can be seen under the microscope as paired threads stainable with basic dyes. The DNA in the chromosome is the main material that becomes stained. As was pointed out in Chapter 1, the chromosomes derive their name from the fact that they take a stain in killed material; the name literally means color bodies. Actually, inside the cell the living chromosomes are practically colorless, very similar to the remainder of the cell, the cytoplasm.

The chromosome is known to all biologists as that part of the organism responsible for the passing of the hereditary traits from one generation to another. Chromosomes are the carriers of the genetic specification that directs one fertilized egg to become a human being, for example, a female with bright red hair, while another fertilized human embryo becomes a male with black hair. The gene responsible for a particular hair color is located somewhere in one of the 23 chromosomes contributed by the egg of the mother or the 23 contributed by the sperm of the father. When a sperm with 23 chromosomes unites with an egg with a similar number, these gametes produce a zygote with 46 chromosomes, which develops into a new human being.

All plants and animals have chromosome numbers characteristic of their given species. A few examples are given in Table 2-1 arranged in ascending order.

Table 2-1. Wide Range of Chromosome Numbers in Animal and Plant Species

Species	Diploid, or Zygotic Number (2n)	Haploid, or Gametic Number (1n)
Ascaris worm	2	1
Fruit fly (<i>Drosophila</i>)	8	4
Trillium	10	5
Peas (used by Mendel)	14	7
Corn (maize)	20	10
Mouse, hog	40	20
Man	46	23
Tobacco	48	24
Cattle	60	30
Horse	60	30
Ass	66	33
Mule	63 (sterile)	?
Wild rhubarb	200	100
<i>Aulacantha</i> species (protozoa)	approx. 1600	approx. 800

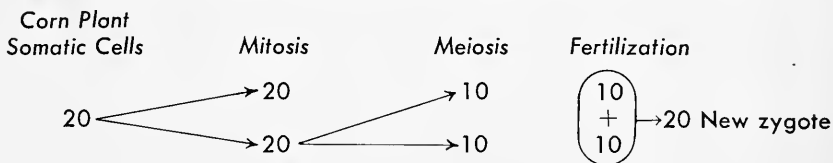
This is not a comprehensive list, but it shows a range of eight hundredfold from a diploid number of 2 in *Ascaris* to approximately 1600 in *Aulacantha*, a species of protozoa.

MITOSIS AND MEIOSIS COMPARED

The type of cell division occurring in all body cells of plants and animals is known as somatic *mitosis*. It occurs in all cell divisions, except in the specialized process of germ cell formation. This process is called *meiosis*, and occurs in the ovaries and testes in animals or in the ovaries and anthers in higher plants. In fungi such as *Neurospora*, meiosis takes place in a special fruiting body called the *perithecium*.

In somatic mitosis the chromosomes are always duplicated before cell division takes place. For example, a corn plant has 20 chromosomes in all the somatic or body cells. Prior to each cell division the 20 chromosomes are duplicated, and then division takes place, resulting in two daughter cells each with 20 chromosomes. If this kind of distribution took place in germ cell formation, it would result in germ cells being formed with 20 chromosomes. The union of two such gametes would form a zygote with 40 chromosomes. In succeeding generations the plant would have 80, 160, etc., with a doubling of chromosomes in each generation. Obviously such a scheme is absurd. Nature has provided for a maintenance of the *same chromosome number* from generation to generation by introducing a different kind of cell division in the formation of the gametes, or germ cells. This is called meiosis and includes a *reduction division*. At this time the somatic number of chromosomes (20 in corn, for example) is halved, so that the gametes possess just half the number (10) found in the somatic cells. The union of two such gametes with the reduced or halved number (10) of chromosomes restores the number in the new zygote to the same number (20) as found in somatic cells of the original corn plant. Since meiosis with its reduction division occurs in the formation of all gametes, the integrity of the chromosome number is maintained. It is diagrammed in Table 2-2.

Table 2-2. Mechanism for Preserving Constant Chromosome Number in Somatic Cells of Corn



The difference between mitosis and meiosis is illustrated in Fig. 2-3. In this diagram the number of chromosomes in all body cells is six ($2n$). Three of these are shown in outline and three as solid. The solid ones represent chromosomes derived from the father and the ones in outline are maternal. The *centromeres*, or spindle attachment regions, are represented as open circles. Note that there are two long chromosomes with median centromeres, two shorter chromosomes with submedian centromeres, and two short chro-

mosomes with subterminal centromeres. In each case there are two of each length, one having been derived from the maternal parent and the other from the paternal. In all somatic mitoses, each chromosome is duplicated before

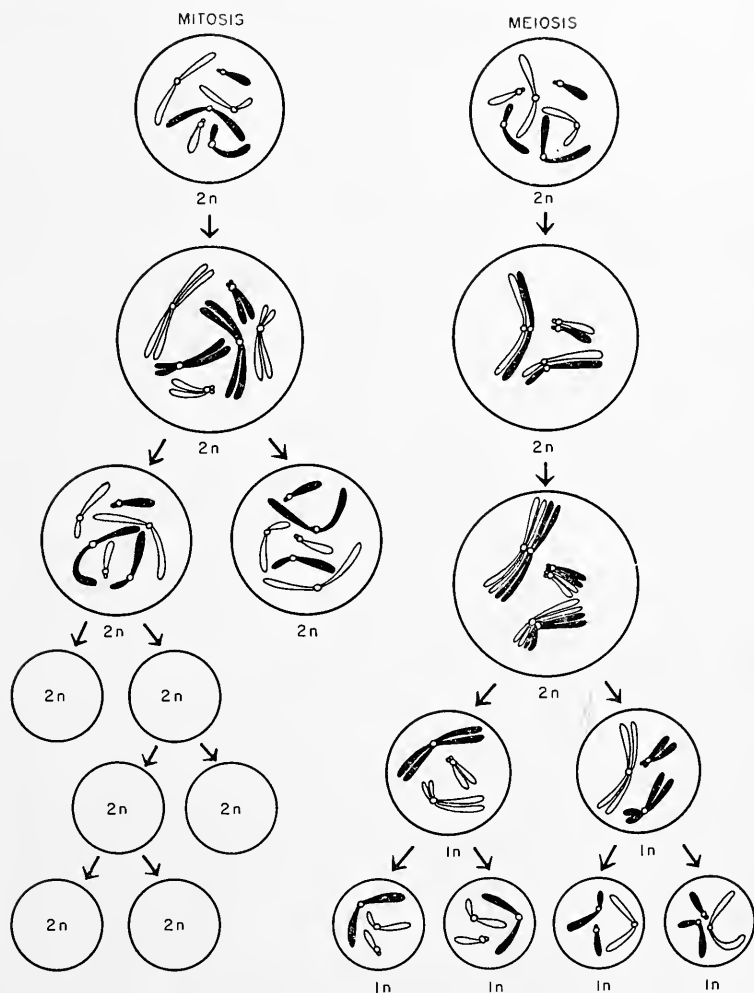
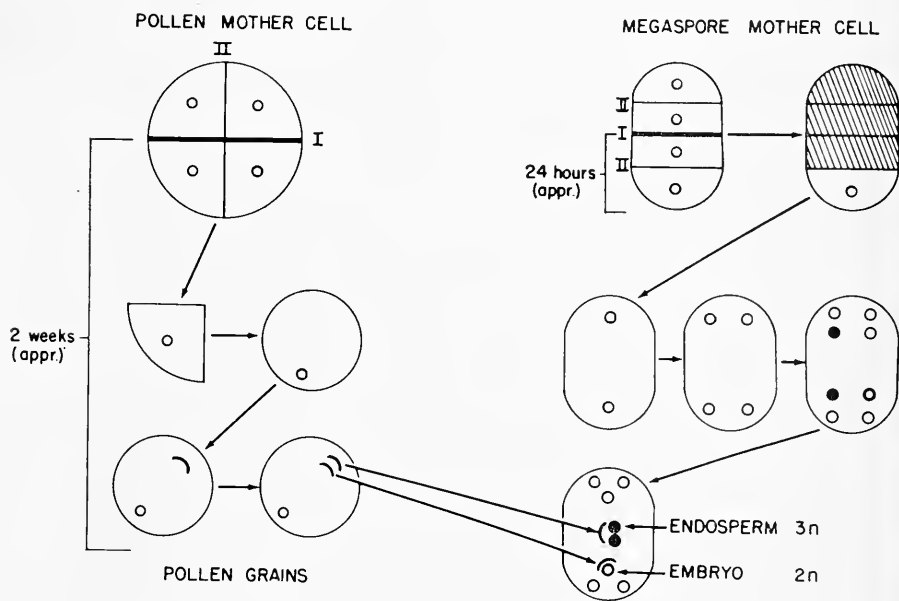


FIG. 2-3 Schematic drawing of chromosomes in mitosis (left) with those at meiosis (right). In mitosis all chromosomes undergo equational division, while in meiosis homologous chromosomes (one derived from father and one from the mother) pair before division, resulting in cells with just half the chromosome number of the original cell.

cell division, resulting in two daughter cells with exactly the same chromosome number as the original cell (6). This is the *diploid* or $2n$ number.

In meiosis, however, the maternally derived chromosomes *pair* with similar chromosomes (homologous) derived from the father. After pairing (*zygotene*

stage), the members of each pair replicate themselves (*diplotene* stage). Each chromosome becomes two *chromatids* held together by an undivided centromere. Then they contract by coiling on themselves and finally separate from each other, in such a way that one member of each pair separates from its mate and goes to an opposite side of the cell. This is the reduction division. The result is the isolation in different parts of the cell of two complete *haploid* (n) sets of chromosomes. During the second meiotic division, which follows immediately, the centromeres divide, permitting each chromatid to separate from its sister chromatid and become a full-fledged chromosome. These new chromosomes now separate from each other in such a way that each haploid



Courtesy of Brookhaven National Laboratory

FIG. 2-4 Schematic representation of microspore and megaspore development in maize.

(n) set is exactly duplicated and four nuclei are formed, each with its own complete set. Now the cell divides into four cells, each with the haploid number (Fig. 2-3). In the higher plants these four cells are either *megaspores* or *microspores* (pollen grains). See Fig. 2-4. In the animals they become eggs or sperm. They contain half the number of chromosomes of the parent that produced them.

MITOSIS AND MEIOSIS IN CORN

Figure 2-5 is a schematic drawing of a corn plant showing the locations where mitosis and meiosis occur. All of the cells of the parts of the

corn plant in outline are produced by mitotic divisions. The only place where meiosis occurs is in the *microsporocytes* in pollen grain formation in the tassel and in similar cells called *megaspocytes* found in the developing ear. The formation of the microspores (the pollen grains) and the megaspores

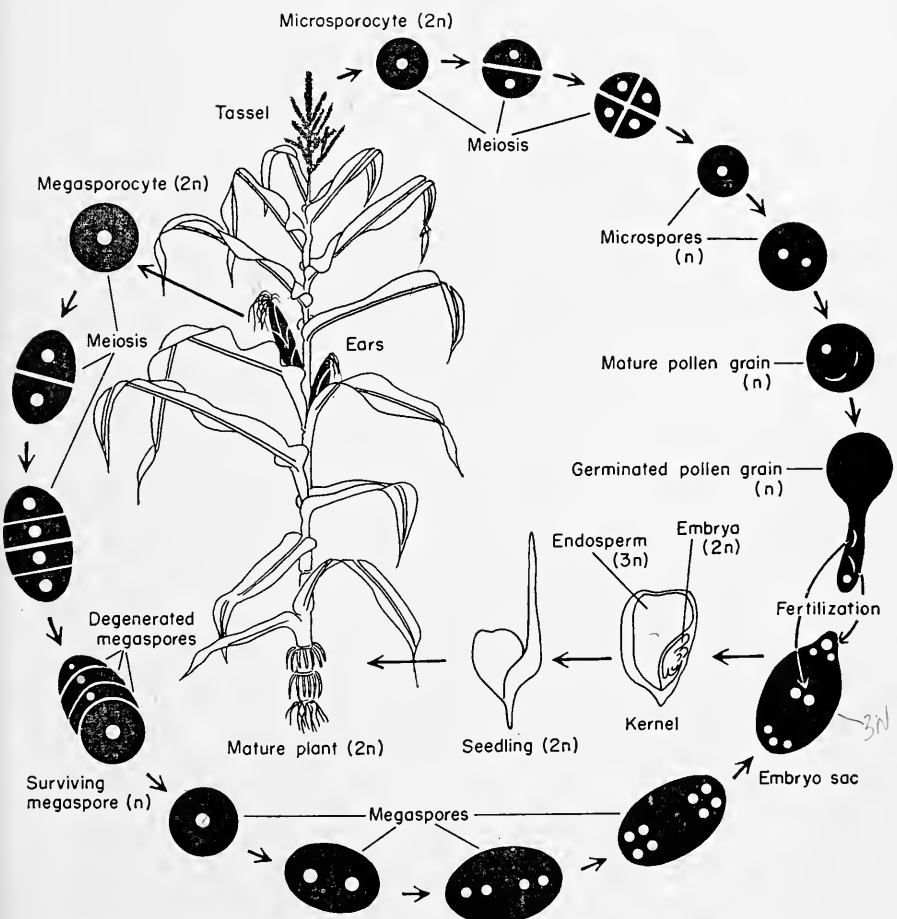
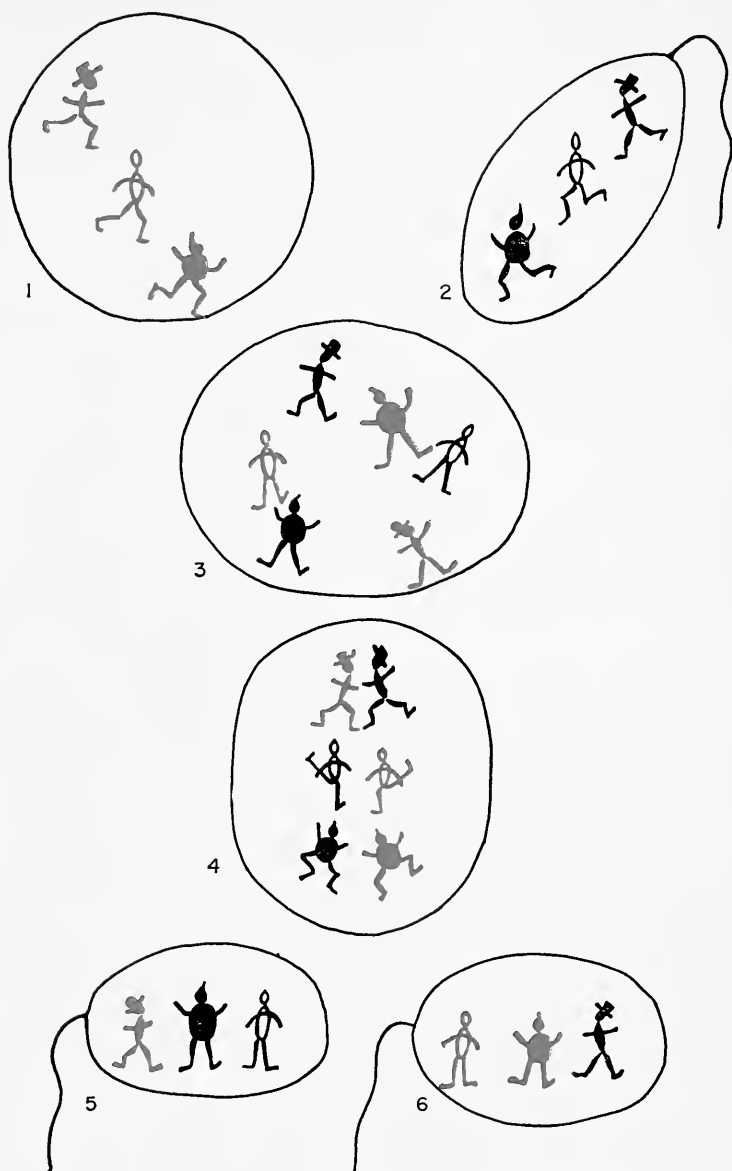


FIG. 2-5 Life cycle in a corn plant. Sporophytic stages are shown in outline, and the gametophytic stage of the life cycle in solid black. Crescent-shaped bodies in the pollen grain are sperm nuclei (male gametes). One of these unites with an egg nucleus to form a zygote which becomes the embryo, and the other unites with two polar nuclei to form the endosperm.

(embryo sac) is known as *microsporogenesis* and *megasporogenesis*, respectively. The tassel and ear, where these occur, are shown solid black in the corn plant (Fig. 2-5).

The diploid part of the corn plant ($2n$) is termed *sporophytic* because it is spore bearing; the haploid (n) part is termed *gametophytic* because it pro-



Courtesy of Charlotte Auerbach; and Oliver & Boyd

FIG. 2-6 "The dance of the chromosomes." (1) The unfertilized egg and (2) the sperm contain one set of chromosomes each. (3) Both sets of chromosomes are present in the fertilized egg and in all cells of the organism that develop from the egg. (4) In preparation for gamete formation, each chromosome finds its partner and pairs with it. Numbers 5 and 6 represent the gametes. One member of each pair of chromosomes has gone into each gamete. Which member—red or black—goes into a particular gamete is left to chance.

duces the gametes. These are the egg nucleus in the female side (the ear) and the sperm nuclei within the pollen grain.

In many plants the division of the generative nucleus to form two sperm is delayed; it occurs within the pollen tube and not in the pollen grain. In corn, as in all grasses, this division takes place in the pollen grain which results in two sperm as shown in Fig. 2-5.

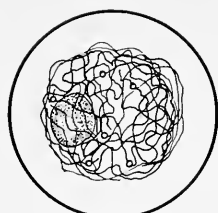
The cycling of the sporophytic and the gametic tissue is known as the *alternation of generations* in plants. In the higher plants most of the life cycle of the plant is sporophytic ($2n$), but in some of the lower forms such as *Neurospora* the greater part of the life cycle is haploid (n) in nature. The diploid phase lasts but a short while, from fertilization within the fruiting body, until meiosis within the same fruiting body, where the reduction division takes place and the haploid chromosome number (n) is restored.

In meiosis, the maternal and paternal chromosomes, which have been wandering about freely in all mitotic divisions, suddenly seek out their partner chromosomes and a pairing takes place. This has been quite aptly referred to as the "dance of the chromosomes" by H. J. Muller, and indeed it resembles one of the old-fashioned square dances where partners meet and separate according to set rules, while the caller gaily directs the procedure. This has been cleverly illustrated by Charlotte Auerbach in her delightful little book *Genetics in the Atomic Age*, from which we reproduce Fig. 2-6. In this dance of the chromosomes the different genes are reshuffled so that the gametes formed represent new combinations of all the chromosomes involved. The larger the number of pairs of chromosomes, the greater the number of possibilities for new assortments in the gametes. Actually the possibilities for new assortments are further increased because partner chromosomes may exchange pieces instead of whole chromosomes. This will be discussed in Chapter 8.

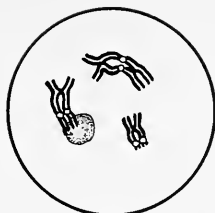
STAGES OF CELL DIVISION

There are certain well-recognized stages that a nucleus goes through in cell division, whether it be a somatic mitosis or the reduction division of meiosis. These stages are the following:

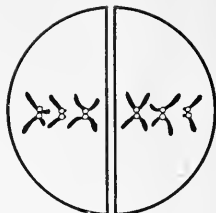
1. *Interphase*, sometimes improperly referred to as "resting stage."
2. *Prophase*, when chromosomes become visible.
3. *Metaphase*, when chromosomes assume position on equatorial plate preparatory to separation.
4. *Anaphase*, a phase after the separation at metaphase, but before they have reached the poles.
5. *Telophase*, a stage after the chromosomes have reached the poles shortly before two new nuclei are formed.



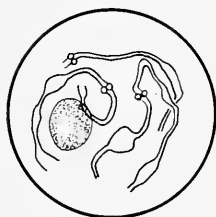
Leptotene



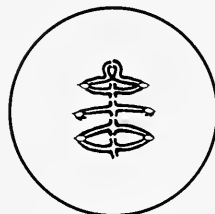
Diakinesis



Metaphase II



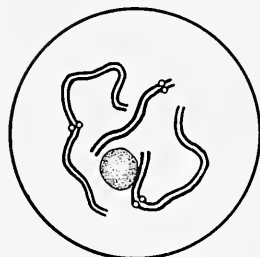
Zygotene



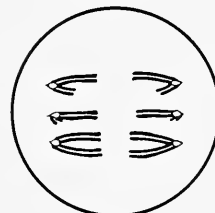
Metaphase I



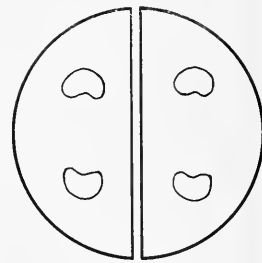
Anaphase II



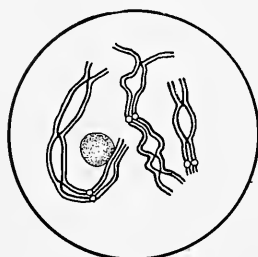
Pachytene



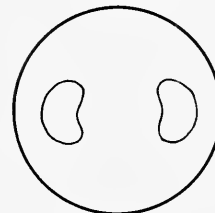
Anaphase I



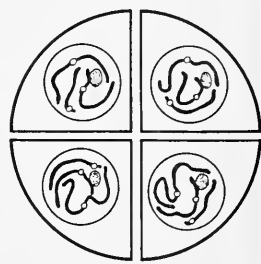
Telophase II



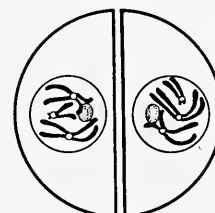
Diplotene



Telophase I



Quartet



Prophase II

FIG. 2-7 Schematic drawing of nucleus undergoing meiosis with three pairs of chromosomes ($2n = 6$; $n = 3$). Meiosis in microsporocyte produces quartets resulting in four pollen grains each, with a haploid number of chromosomes, $n = 3$. Chromosomes are visible as threads at the leptotene stage, and become paired at the zygotene, with pairing complete at the pachytene. The nucleolus is visible through diakinesis. Chiasmata are visible at the diplotene and diakinesis, where chromosomes are much shortened. Chromosomes undergo division at metaphase I, where reduction of chromosomes takes place. The rest of the stages are self-explanatory.

The chromosomes have a characteristic appearance in each of these five stages, as shown in Fig. 2-7. It should be borne in mind that these are stages in a continuous process, from one interphase nucleus to the new interphase nuclei in the newly formed cells.

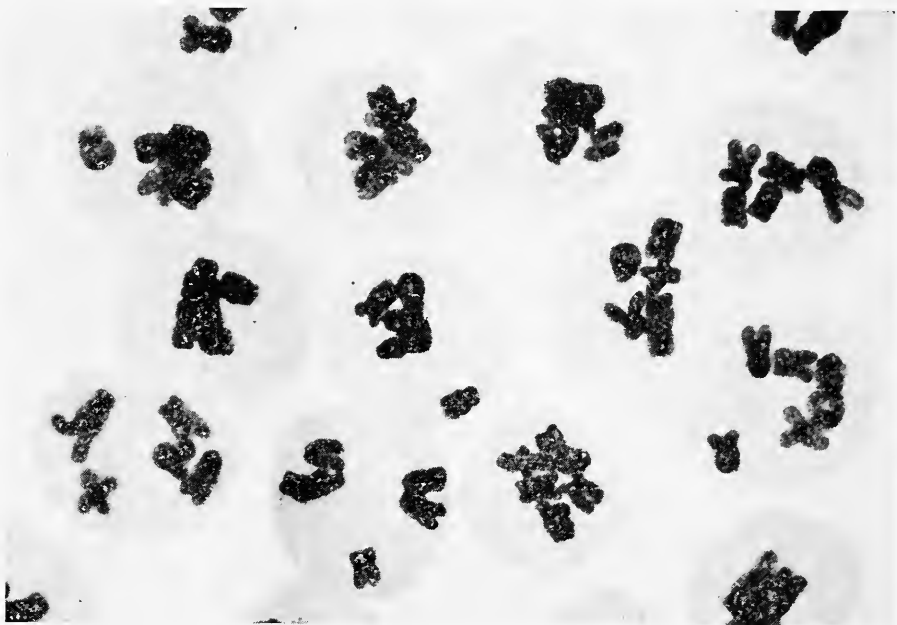
Anyone who has seen a moving picture of cell division is well aware that a cell division is something dynamic—a living, pulsing process. Nothing is static. The period of the so-called resting nucleus is not really resting, but is one of the most active periods in the cell division during which, it is thought, the DNA molecules are being duplicated. The word interphase is a better term for this stage and is now more commonly used.

Although it is important to be familiar with the appearance of the various stages of cell division, we must not lose sight of what is accomplished by both mitosis and meiosis. In mitosis, the cells are duplicated faithfully and with an exactness that almost defies description. The cell goes through this elaborate process, resulting in two daughter cells exactly like the mother cell. Each chromosome and each gene is duplicated faithfully with a precision that by comparison makes the manufacture of a many-jeweled watch seem a fumbling and clumsy operation. Chromosomes undergo division countless times without a mistake. At rare intervals a mistake is made. Then the copy is not an exact duplicate of the original. What then occurs is known as a *mutation*. This happens at rare intervals—perhaps once in a hundred thousand, or once in a million times. Without these mutations there would have been no evolution, with the great variety exemplified in the plant and animal kingdoms, and no science of genetics whatever.

In meiosis the genes and chromosomes are shuffled and dealt out to the newly formed germ cells much as a deck of cards is dealt out to four individual hands. Let the black cards represent the paternal chromosomes and the red ones the maternal ones, with the different numbers representing different genes. We get some idea of the diversity that we might expect when the actual chromosomes and genes are shuffled at the dance of the chromosomes and dealt out to the new gametes or germ cells. Actually, the process is more complicated than dealing a deck of cards, as there are many more chromosomes, with perhaps thousands of genes in each. It is little wonder then that no two individuals (except identical twins) in sexually propagated species are exactly alike. In asexually propagated species the new individual is exactly like the progenitor, since there has been no opportunity for a reshuffling of the chromosomes. Many horticultural plants are good examples of asexual propagation. The newly propagated plant has chromosomes and genes the same as the parental types, and is alike in every detail, barring a somatic mutation. In case of a somatic mutation the new type can be propagated and will maintain itself as faithfully as the parental type, resulting in a new horticultural variety.

MEIOSIS IN TRILLIUM

Let us return to the complex flower bud of *Trillium* in Fig. 2-1. A great many cells are represented, and this is only a thin slice through the bud. Actually it would take ten such slices to equal the thickness of the page you are now reading. The cells seen in this figure would have to be multiplied by many thousands, perhaps more than a million, to represent the total number of cells in a *Trillium* plant, and each cell has arisen from a pre-existing cell



Courtesy of A. H. Sparrow; and Brookhaven National Laboratory

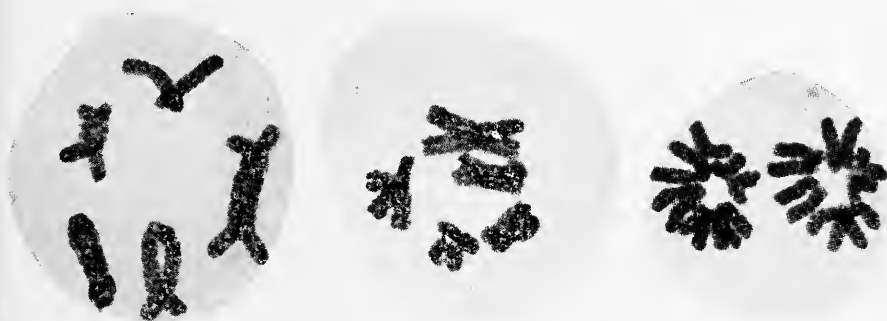
FIG. 2-8 Microsporocytes in *Trillium* showing all cells in the same stage of division, metaphase I. Synchronization of stages of meiosis in this plant, in addition to extremely large chromosomes, make it an excellent cytological tool for radiation research.

by the process of mitosis, already described. In the flower bud can be seen the ovary (in the center) and the six anthers surrounding the ovary.

If we enlarge a minute speck of one of the six anthers, say a portion about as big as the period at the end of this sentence, and magnify it sufficiently, we would get a picture like that in Fig. 2-8. In *Trillium* and in most species of flowering plants the meiotic divisions are synchronized, so that the chromosomes go through their divisions together. This is how a figure such as Fig. 2-8 is possible, showing all the cells in the metaphase stage, in which the chromosomes are lined up on the equatorial plane. The gigantic size of the chromosomes and the fact that the divisions in *Trillium* are synchronized explain why

this is an excellent plant for cytological investigations. Arnold Sparrow and his colleagues at the Brookhaven National Laboratory have used it extensively and effectively in studying radiation effects.

If we magnify a single cell of *Trillium* in the metaphase stage, the chromosomes can be studied in considerable detail. Such cells appear in Fig. 2-9*a*, *b*,



Courtesy of A. H. Sparrow; and Brookhaven National Laboratory

FIG. 2-9 Enlarged microsporocyte in *Trillium* meiosis I. (Left) Early metaphase: chromosomes are paired, but four strands do not show division. (Middle) Metaphase: chromosomes are divided in preparation for second division. (Right) Second metaphase, where four microspores will be formed with five chromosomes each.

and *c*. The double nature of the chromosomes is clearly seen in *b* and *c*, respectively.

LIFE CYCLE IN ANIMALS

The life cycle in animals is similar to that in plants, as exemplified in the diagram of the life cycle of a corn plant (Fig. 2-4). The body cells of animals are diploid ($2n$), having derived half of their chromosomes from the father and half from the mother. A typical life cycle for animals is shown in a schematic diagram for man, from fertilization to the production of sperm and ova that unite, resulting in a zygote or a new generation (Fig. 2-10). The main difference between animals and higher plants is the elimination of the gametophytic generation in animals. In plants there are one or more divisions in the haploid tissue before the gametes are produced, while in animals the gametes (the sperm and eggs) are produced directly as a result of meiosis.

A study of Fig. 2-10 reveals that the body cells are $2n$ (diploid) in number and that the n (haploid) number exists only after the first meiotic division. In the female, the first meiotic division of the *primary oöcyte* results in one *secondary oöcyte* and a *polar body*. At the second meiotic division, the *secondary oöcyte* divides to produce the *ovum* (the female gamete) and another *polar body*. This makes three *polar bodies* in all, since the *polar body* formed at meiosis I divides at the second meiotic division to produce two *polar bodies*.

In the male the *primary spermatocyte* ($2n$) in the testis divides at meiosis I

to form two *secondary spermatocytes*, each with the reduced chromosome number. The reshuffling of paternal and maternal chromosomes takes place at meiosis I, even though the chromosomes at this stage have been duplicated preparatory to the second division when the four *spermatids* are formed. Each one of the spermatids becomes a mature *sperm*, the *male gamete*. The proc-

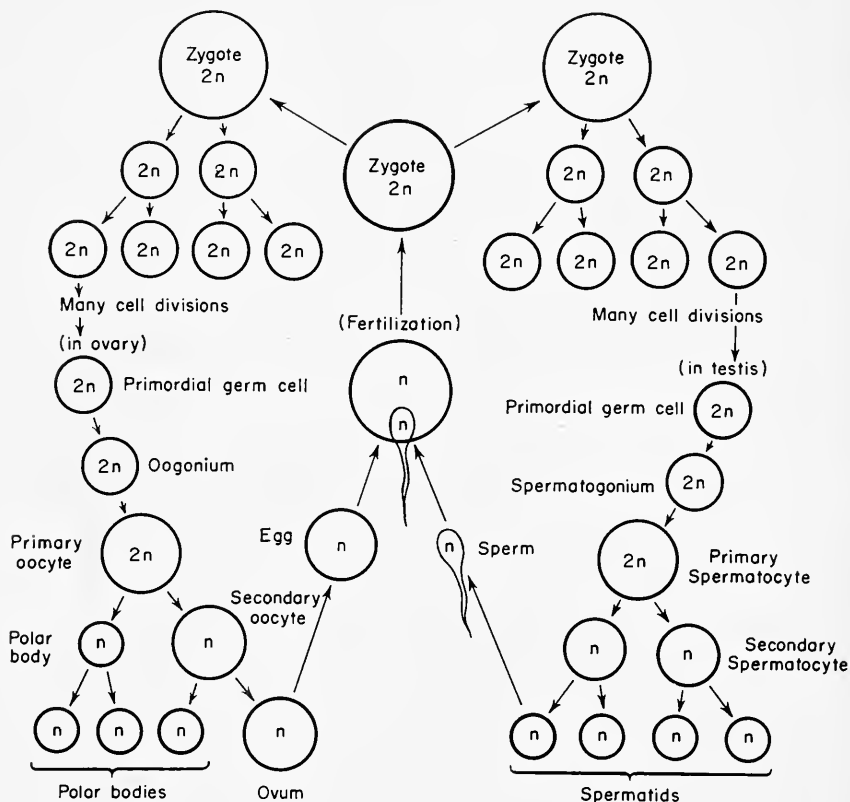


FIG. 2-10 Schematic diagram of the life cycle in man. Body cells (somatic) all contain diploid number of chromosomes. In woman (left side of diagram), reduction division takes place in the ovary in a primary oöcyte; in man it takes place in the testis in a primary spermatocyte, resulting in the haploid sperm which unites with an egg from a primary oöcyte and restores the diploid number.

esses by which sperm and ova are produced are known as *spermatogenesis* and *oögenesis*, respectively. The divisions of the germinal tissue preceding the reduction division are known as the *spermatogonial* and *oögonial* divisions. *Spermatogonia* and *oögonia* are made up of diploid cells. The haploid sperm and eggs result from the meiotic division of primary spermatocytes and primary oöcytes within the spermatogonium and oögonium, respectively.

HISTORICAL ASPECTS OF THE CHROMOSOME
THEORY OF HEREDITY

In the interval between the publication of Mendel's paper and the rediscovery of his classic researches by Correns, De Vries, and Tschermak, a number of competent cytologists were studying the cytological behavior of chromosomes. By 1875 Strasburger had described chromosomes, and Hertwig had proved that fertilization involved the fusion of the two parental nuclei found in the egg and sperm. By 1882 Flemming had described the longitudinal splitting of the chromosomes. In 1883 Van Beneden announced the principle of genetic continuity of chromosomes and reported the occurrence of chromosome reduction at germ cell formation.

Between 1884 and 1888, identification of the cell nucleus as the basis for inheritance was independently reported by Hertwig, Strassburger, Kolker, and Weismann. In 1887 Weismann proposed an all-embracing theory of chromosome behavior during cell division and fertilization and predicted the occurrence of meiosis. Roux suggested that the linearly arranged qualities of the chromosomes were equally transmitted to daughter nuclei at meiosis. In 1890 the numerical equality of paternal and maternal chromosomes at fertilization was established by Boveri in Germany and Guignard in France.

In 1892 the publication of Weismann's book *Das Keim Plasma* (The Germ Plasm), emphasized meiosis as an exact mechanism for chromosome distribution. In 1898 Fleming reported the chromosome number of man to be 24 pairs. (Recent researches have shown the correct number to be 23.) In 1902-03 Sutton pointed out the interrelationships between cytology and Mendelism, closing the gap between cell morphology and heredity.¹

CONCLUDING REMARKS

All plants and animals are composed of cells. The hereditary determiners, the genes, are contained in the chromosomes in the nucleus, which divides with great precision when new cells are formed. The chromosomes are divided equationally at mitosis to form new somatic or body cells. At meiosis, during the reduction division, homologous chromosomes pair before division, and the pairs separate so that each new cell contains just half the number of the original cell. Meiosis occurs at germ cell formation: in the testes and ovaries in animals, in anthers and ovaries in higher plants.

Meiosis is the process by which the reshuffling and distribution of the genic material to the newly formed gametes is accomplished. Such a distribution was postulated by Mendel in 1865, before he knew of the existence of chro-

¹The foregoing historical statements are extracts from a chronology published by Gabriel and Fogel in *Great Experiments in Biology* and are used with permission of the authors and the publisher.

mosomes or their behavior at meiosis. The researches of many cytologists have verified the original assumption of Mendel and have shown that the chromosomes are the carriers of heredity.

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PROBLEMS

2-1. Define the following terms:

anaphase	Mendel's law
centromere	metaphase
chromonema	microsporogenesis
chromosome	mitosis
chromatin	oögenesis
diplotene	ovary
DNA	pollen
egg	primary spermatocyte
embryo	prophase
endosperm	reduced number of chromosomes
equatorial plate	self-duplicating
gametic	somatic chromosome number
germ plasm	sperm
homologous	spermatogenesis
interphase	spermatogonial
karyolymph	sporophytic
megasporogenesis	telophase
meiosis	zygotene

2-2. Identify the following scientists, giving a major contribution of each, with an approximate date.

Correns, Carl	Tschermak, E. von
De Vries, Hugo	Weismann, A.
Sutton, W. S.	

2-3. In corn, how many chromosomes are in the following?

root tip	embryo
pollen mother cell	endosperm
pollen grain	pericarp
anther wall	aleurone layer
sperm nucleus	leaves

2-4. In human beings, how many chromosomes are in the following?

body cells	fertilized egg
primary spermatocyte	primary oöcyte
spermatogonial cells	ovum
spermatid	polar body
sperm cell	secondary spermatocyte

2-5. In the mouse how many chromosomes are in the following?

somatic cell	secondary spermatocyte
primary spermatocyte	spermatid

2-6. How many mature spermatozoa are produced from 100 primary spermatocytes?

2-7. How many eggs are produced from 100 primary oöcytes?

2-8. How many polar bodies from 100 primary oöcytes?

2-9. Fig. 2-1 shows a bud of *Trillium* with six anthers. It has been estimated (by Miss Virginia Pond of the Brookhaven National Laboratory) that each anther contains approximately 5000 pollen mother cells. How many mature pollen grains would be expected from a single anther? From an entire flower?

One Gene Difference

THIS CHAPTER is perhaps the most important for an understanding of the mechanism of Mendelian analysis. It is similar to multiplication tables in the study of mathematics.

We have already seen in Chapter 1 examples of characters whose inheritance is determined by a single gene. The term "gene" is used to designate the hereditary determiner, and it is synonymous with the term "element" of Mendel.

Mendel's great contribution to an understanding of the mechanism of heredity was that he realized that the hereditary differences he saw were caused by particulate determiners. These he called elements. We now call them genes. The name is not important, but the concept that the visible characters were due to particulate determiners is the essence of Mendel's law. There were other attributes which were necessary to explain results, but the concept of the discrete hereditary determiners was what made Mendel's law different from the blending concept then in vogue. We can properly say that Mendel discovered the gene. It was not so named until many years later, but Mendel discovered it just as truly as Columbus discovered America, which also was not so named until many years later.

These hereditary determiners are distributed at random when the germ cells are being formed. They are not altered in any way by their association with other genes in the hybrid organism. They separate cleanly. (There are a few exceptions which will be discussed in Chapter 22). A gene emerging from a hybrid individual is in no way different from the same gene of a pure line. As an example of how this operates, we can do no better than to return to Mendel for our explanation. He found that when he crossed a tall pea by a dwarf pea, the hybrid was tall like one of the parents, the tall one. In fact, by looking at the plants it was not possible to tell any difference between the

hybrid and one of the parents. Since the tall parent seemed to "dominate" the appearance of the hybrid, he called it dominant. The opposite of this, the dwarf condition, was called the recessive. In Mendel's own words:

Henceforth in this paper those characters which are transmitted entire, or almost unchanged in the hybridization, and therefore in themselves constitute the characters of the hybrid, are termed *dominant* and those that become latent in the process *recessive*. The expression "recessive" has been chosen because the characters thereby designated withdraw or entirely disappear in the hybrids, but nevertheless reappear unchanged in their progeny. . . .

An equally good example of this can be found in the corn plant, which has contributed much to our knowledge of genetics. In corn there are definite dwarfs which are inherited as simple recessives.

It is necessary at this point to introduce a few more technical terms. The parents of any cross are designated by the capital letter P. The first generation hybrid between two parents is called the first filial generation, abbreviated to F_1 . A mating of two F_1 hybrids, or self-fertilization of the F_1 , as is possible in plants, produces the F_2 generation. Let us see how this self-fertilization operates when the dwarf and the tall peas are crossed, or dwarf and tall corn (Fig. 3-1).

Parents (P).....	D/D tall	d/d dwarf
Germ cells (gametes).....	D	d
F_1 hybrid.....	D/d tall because D is dominant	

We have used the designation of the tall (D/D) and dwarf (d/d) types to conform to present-day usage among geneticists. The genotypes are written D/D and d/d , rather than DD and dd , to indicate the presence of the different alleles in different but homologous chromosomes. Actually Mendel did not assign a special letter to represent the characters with which he worked, but used the letters A , B , or C to designate any character under study.

Note that the genotypes of the parents are written D/D and d/d rather than a D or d . This is because the parents have two genes, alleles of the one gene being studied. All plants and animals have their chromosomes in pairs, one member of each from the father and one from the mother. The genes or hereditary determiners are located in the chromosomes, and these chromosome pairs are separated at the reduction division of meiosis, as we have seen in the previous chapter. Mendel knew nothing of chromosomes and their behavior at meiosis. It is remarkable that he designed a correct conceptual scheme for the distribution of these hereditary determiners when the germ cells were formed.

MENDEL'S EXPERIMENTS PLANNED WITH CARE

Mendel chose his experimental material, the common garden pea, with great care. Regarding a choice of material he states:

The value and utility of any experiment are determined by the fitness of the material to the purpose for which it is used, and thus in the case before us it can not be immaterial what plants are subjected to experiments and in what manner such experiments are conducted.

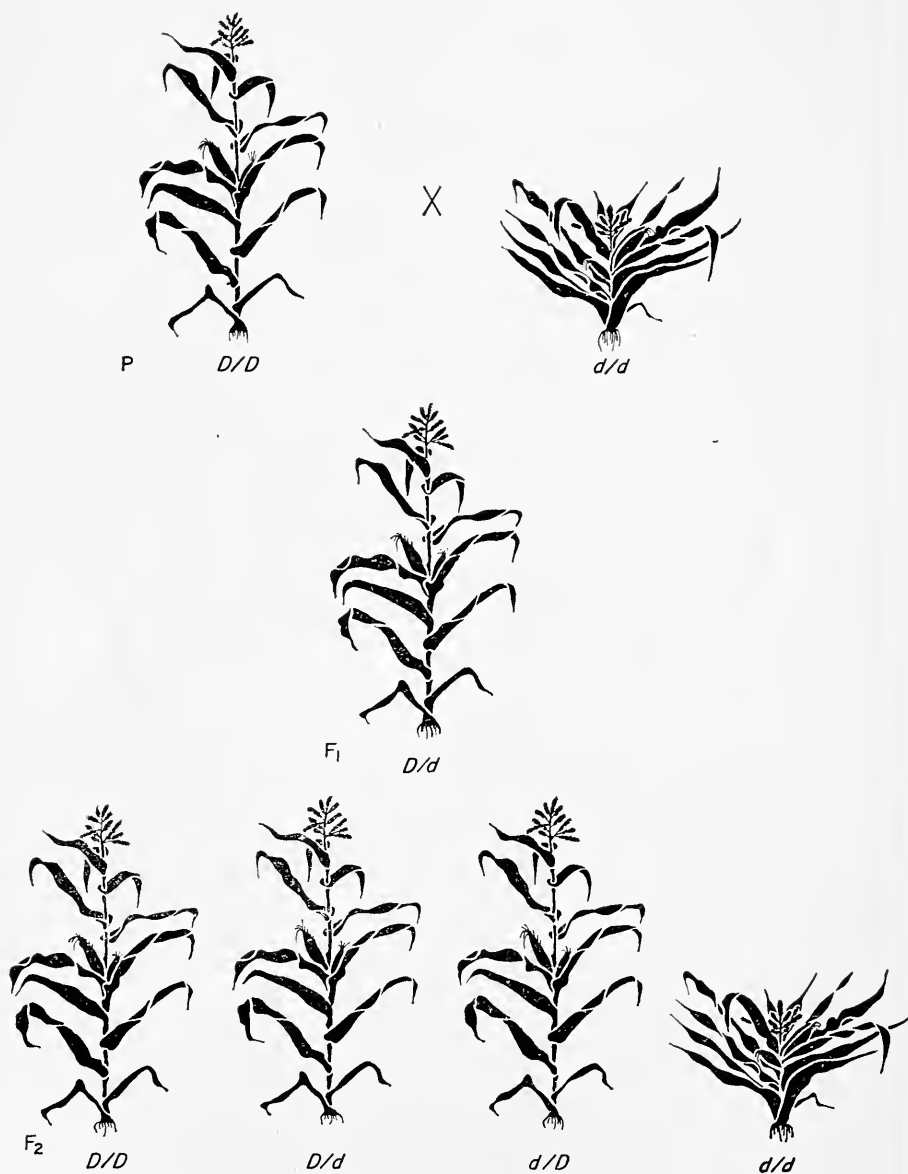


FIG. 3-1 Inheritance of single gene difference in maize. Tall (D/D) crosses with dwarf (d/d) produce tall phenotype in F_1 , which upon self-pollination produces F_2 consisting of 3 tall:1 dwarf (bottom line).

The experimental plants must necessarily (1) possess constant differentiating characters, and (2) the hybrids of such plants must, during the flowering period, be protected from the influence of all foreign pollen, or be easily capable of such protection.

It is evident that the common pea, *Pisum sativum*, fulfilled adequately both of these requirements. It is a naturally self-pollinated plant. Consequently the stocks that Mendel used were pure lines, homozygous, and not mixed in their heredity, heterozygous. It would have been virtually impossible to design such precise laws of heredity if he had not worked with pure lines.

Another advantage of a self-pollinated organism was that, once the first generation hybrid was made, no further hand selfing was necessary to obtain the F_2 generation. The plants would pollinate themselves and each plant would produce many pods and as many as a hundred seeds, *by self-pollination*.

One of the reasons for Mendel's success was the care with which he planned his experiments. He selected a self-fertilized plant with constant differentiating characters. He studied small differences conditioned by a single gene. If he was "lucky," as has sometimes been implied, his "luck" consisted in careful planning, meticulous workmanship, keen observation, and hard work.

Now let us see what happens when the gametes of the F_1 plant are produced. Here Mendel analyzed correctly and precisely what takes place. The F_1 has the genetic constitution, the genotype, D/d , and is tall in its appearance, the *phenotype*. The tall parent D/D and the F_1 hybrid D/d have the same phenotype, but different genotypes.

It is evident that pure lines, represented by these two parents, P , each can produce only one kind of germ cell or gamete, either D or d ; the F_1 hybrid can and must produce two types, D and d . Mendel realized that these would be produced in equal numbers, and the D and d would be just as pure gametes as if they had come from pure lines. In other words, they were completely unaffected by their brief association with alleles of the opposite kind.

The gametes D and d are produced in equal numbers, each having a probability of one half. Both male and female gametes are of the two kinds, D

Table 3-1. Gametes and Progeny Produced by Self-Pollinated $F_1 = D/d$ (Tall Phenotype)

♂ gametes	D	d
♀ gametes		
D	D/D tall will breed true	D/d tall phenotype will segregate
d	D/d tall phenotype will segregate	d/d dwarf will breed true

and *d*. The results are easily understood if the male and female gametes are placed on different axes of a checkerboard, or Punnett square, so named because it was first used by an early English geneticist, R. C. Punnett.

The possible combinations are shown in Table 3-1.

The diagram illustrates Mendel's law of segregation. It also shows the consequences of dominance. The F_2 consists of three-fourths dominant (tall) and one-fourth recessive (dwarf). However, only one-third of the tall plants breeds true; the other two-thirds are exactly like the F_1 and will segregate in the next generation. The recessive is a pure line and will breed true.

PROBABILITIES UNDERLYING GENETIC SEGREGATION

The proportions produced by segregation can be expressed in another way. Since the probability for each gamete formed is $\frac{1}{2}$, the resulting segregation can be expressed by multiplying the probabilities of the different gametes to give the results found in Table 3-2. Note that (2) and (3) are the

Table 3-2. Probabilities of Gametes Produced by F_1 , and Proportions of Genotypes in F_2

	Female \times Male Gametes	Probabilities	Proportions of F_2 Populations
(1)	$D \times D$	$\frac{1}{2} \times \frac{1}{2}$	$D/D: \frac{1}{4}$
(2)	$D \times d$	$\frac{1}{2} \times \frac{1}{2}$	$D/d: \frac{1}{4}$
(3)	$d \times D$	$\frac{1}{2} \times \frac{1}{2}$	$d/D: \frac{1}{4}$
(4)	$d \times d$	$\frac{1}{2} \times \frac{1}{2}$	$d/d: \frac{1}{4}$

same; it makes no difference whether they received a *D* or *d* from the male or female gamete.

Any student who has had a course in elementary algebra will notice the striking similarity between this segregating progeny and the expansion of the binomial $(a + b)^2$ which is $a^2 + 2ab + b^2$.

If we should write $(a + b)^2$ the way geneticists usually describe a mono-

Table 3-3. Checkerboard Showing Results of $(a + b)^2$

	a	b
a	$aa(a^2)$	ab
b	ab	$bb(b^2)$

genic segregation by use of the checkerboard, we would set up a checkerboard as in Table 3-3. This would give $a^2 + 2ab + b^2$.

If we substituted the letters D and d for a and b we would get the results shown in Table 3-4. It makes no difference whether we write the first term D^2

Table 3-4. Checkerboard Showing Results of $(D + d)^2$

♀ gametes ↘ ♂ gametes	D	d
D	$D/D(D^2)$	D/d
d	D/d	$d/d(d^2)$

or D/D , as it is customarily written by geneticists, or the last term d^2 or d/d . Consequently, the basic law of segregation for a one gene difference is the same as the expansion of the binomial $(a + b)^2$. If we substitute the probabilities $\frac{1}{2}$ and $\frac{1}{2}$ for the values D and d we get $(\frac{1}{2})^2 + 2(\frac{1}{2} \times \frac{1}{2}) + (\frac{1}{2})^2$, which gives us the $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ or the familiar 1:2:1 genotypic ratio for a one gene difference.

If dominance is present, as it is in the case of the tall peas or tall corn, it is not possible to tell whether a tall plant is homozygous, D/D , or heterozygous, D/d . In the absence of dominance the F_1 is different phenotypically from



FIG. 3-2 Segregation of 1:2:1 in soybeans. The recessive type is golden (g/g). Homozygous green plants, G/G , are darker green than heterozygous plants, G/g , giving a 1:2:1 ratio.

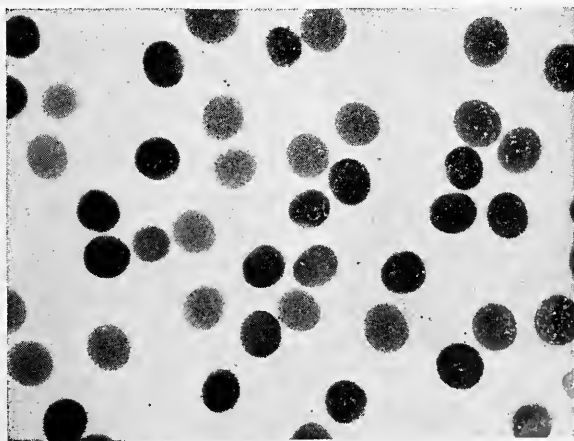
one locus since it has two homologous chromosomes. The full genotype of a brown-eyed *Drosophila* would look something like Fig. 3-3.

Obviously, it is neither practical nor desirable to write the full genotype of every fruit fly in genetic studies. We are concerned only with the characters in which the individuals differ, so that the pedigree of a brown-eyed fly is written as *bw/bw* if it is the one gene being studied. If it is crossed with a scarlet-eyed fly, for example, then an allele at the scarlet locus in another pair of chromosomes must be shown. In this case the genotype of brown becomes *bw/bw +/+*. Conversely the pedigree of scarlet is written *+/+ st/st*. The brown fly produces *bw+* and the scarlet fly *+st* gametes. The union of these gametes will give a fly with the constitution *+/bw +/st*. Each of the recessive alleles in this individual is paired with a wild-type allele (+) and hence the F_1 fly has red eyes similar to the wild-type phenotype. The segregation in the F_2 will be discussed in Chapter 4.

Mendelian analysis is based on a random segregation of the gametes in the F_1 individual. We shall see the consequences of segregation of two or more genes in Chapter 4, and also exceptions due to linkage in Chapter 8.

GENETIC DIFFERENCE AMONG GAMETOPHYTES

In higher plants or animals, with few exceptions, it is not possible to observe any differences in the gametes, but these differences are inferred from the breeding results. A notable exception occurs in the corn plant, rice, sorghum, and perhaps others, where a difference can be observed in the gametophytic generation in the pollen grain. This is the gene for waxy endo-



Courtesy of B. Varma and James Riopel, University of Virginia

FIG. 3-4 Segregation for waxy (*wx*) and normal (*Wx*) pollen grains. Normal grains stain blue with iodine, while waxy grains stain red.

sperm, which is also observable in the pollen, the male gametophyte with n number of chromosomes (haploid). In corn the gene for waxy endosperm is located in chromosome 9. Kernels homozygous for wx/wx (endosperm $wx\ wx\ wx$) possess a different kind of starch than Wx/Wx or Wx/wx kernels. Both Wx/Wx and Wx/wx kernels have a starch that stains blue with iodine, while the starch of wx/wx kernels stains red. The same staining reaction applies to the pollen (Fig. 3-4). Since Wx grains stain blue and wx pollen grains stain red with iodine, it is possible to observe the gametophytic generation directly. It has been found that Wx and wx pollen grains are produced in approximately equal numbers, which had been inferred from the breeding tests in which F_1 plants produced $\frac{3}{4}$ $Wx/-$ kernels and $\frac{1}{4}$ wx/wx kernels in the F_2 .

In all cases except the waxy gene in higher plants, we are limited to a study of diploid organisms and hence cannot study directly the segregation in the gametophytic (n) generation. No such limitations exist for the lower plant forms in which characters can be observed in the haploid generation possessing only one set of chromosomes.

DODGE'S EARLY STUDIES OF INHERITANCE IN NEUROSPORA

The classic work of B. O. Dodge (Fig. 3-5) in the late 1920's and early 1930's at the New York Botanical Garden demonstrated genetic differ-



Bachrach

FIG. 3-5 B. O. Dodge, who first determined the genetics of *Neurospora*.

ences in a bread mold, *Neurospora*. In this case the organism is mostly haploid, except for a brief period following the fusion of two different mating types of mycelia, the long, threadlike strands comprising the main body of the mold. Following this fusion, or fertilization, a fruiting body, the *ascus*, develops and the *ascospores* are confined in small envelopes containing eight spores arranged in a manner shown in Fig. 3-6. The *ascospores* are arranged linearly so that the product of a single meiosis can be observed. Hence it is possible to see clearly *all* the resulting spores from a single meiosis. It is not necessary to make a statistical evaluation, as is done in all higher organisms, even the number of wx and Wx pollen grains where the different gametophytic genotypes can be observed directly. Dodge found that, when two types were

crossed, the eight ascospores were divided equally (four and four) between the different parental types. The pioneering work of Dodge demonstrated the excellence of *Neurospora* as a genetic tool, which later was used so effectively by George W. Beadle and Edward L. Tatum in demonstrating biochemical genetics.

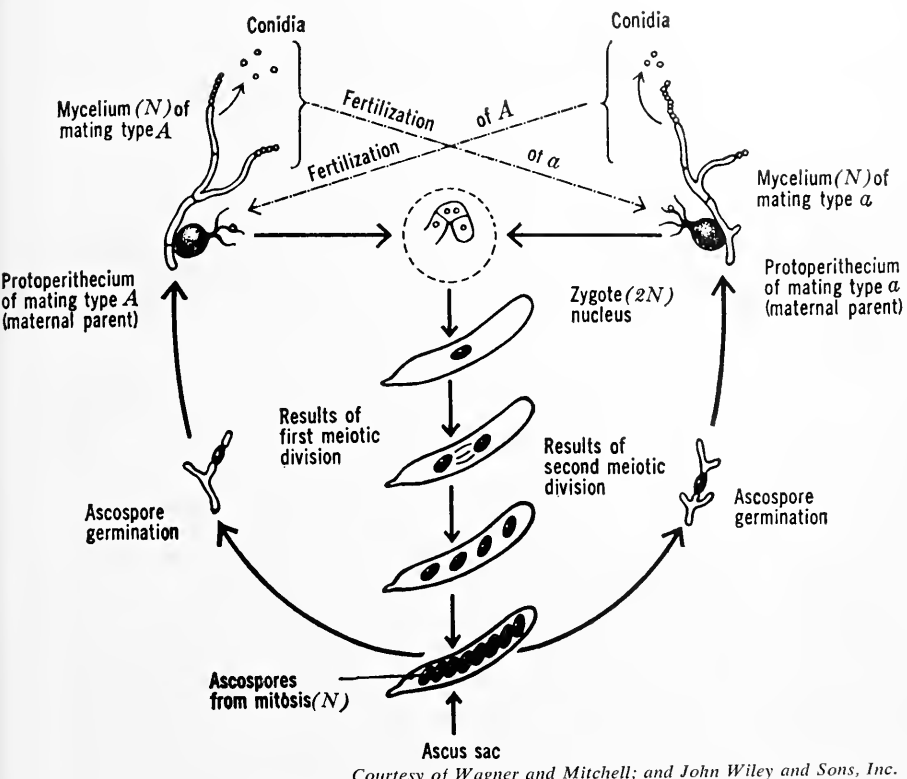


FIG. 3-6 Life cycle in *Neurospora*.
Courtesy of Wagner and Mitchell; and John Wiley and Sons, Inc.

SEGREGATION FOR RED HAIR COLOR IN MAN

Let us return to the inheritance of hair color mentioned in Chapters 1 and 2. We use this because everyone is familiar with red hair color, and most are aware of the striking differences within families. Suppose a redheaded woman married a man with dark hair. For the sake of illustration, we will assume that he received a gene for dark hair from both his father and mother, i.e., he was pure, or homozygous, for dark color. Such a marriage is shown in Table 3-5.

Table 3-5. Result of Marriage of Two Homozygous Types

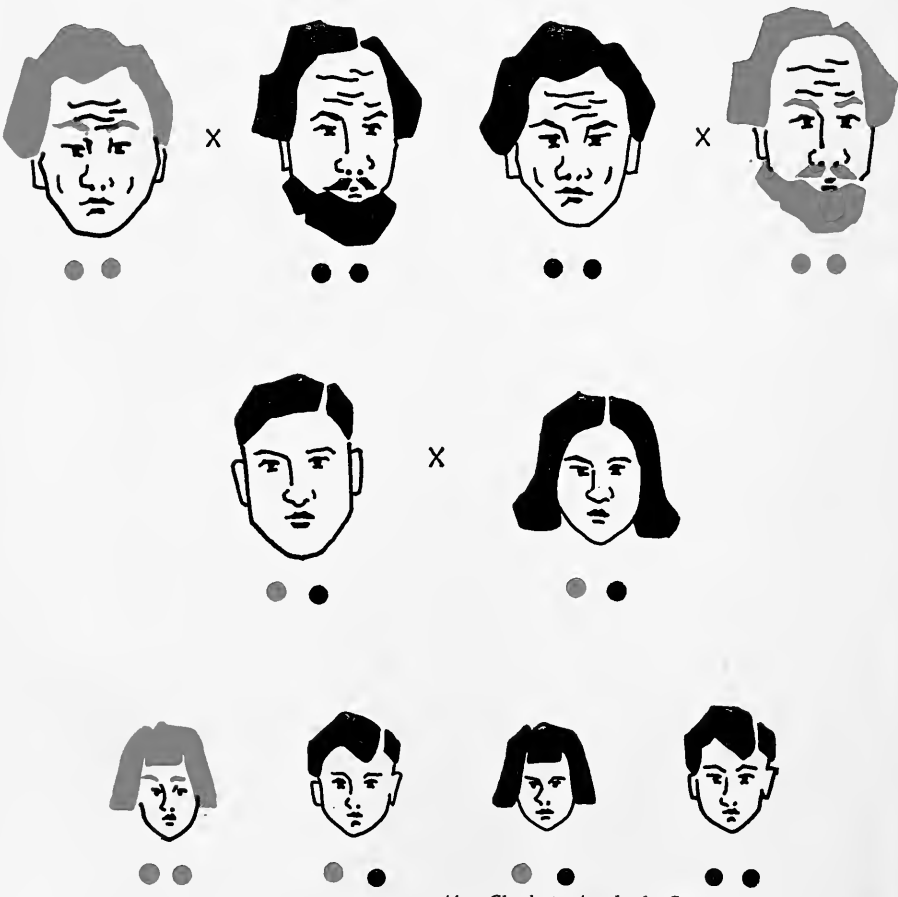
		Woman r/r (red hair) \times Man R/R (dark hair)	
Gametes.....	r		R
F_1		R/r dark	

A marriage of two R/r people gives proportions as shown below:

- $\frac{1}{4} R/R = \text{dark}$
- $\frac{1}{2} R/r = \text{dark}$
- $\frac{1}{4} r/r = \text{red}$

Since R/R and R/r are indistinguishable (phenotypically alike) three fourths of the children should have dark hair and one fourth red hair (Fig. 3-7).

You will recall that, of the two segregating families in Chapter 1, one had



After Charlotte Auerbach; Courtesy of Oliver & Boyd

FIG. 3-7 Schematic diagram of inheritance of red hair in man. Two grandparents are homozygous for R/R , two for r/r . The parents are each R/r . Three children are $R/-$ (non-red), while one is red r/r .

a segregation of 1 dark : 1 red, while the other had a segregation of 2 dark : 1 red, where a 3:1 ratio was expected. In Chapter 5 we will discuss how much of a deviation from an expected ratio may be tolerated without invalidating the proposition that an independent assortment is operating.

The family of the four red-haired girls in Chapter 1 had only r alleles. The father and mother being r/r could produce only gametes that were r , and the union of any two of these would give r/r individuals all red.

TESTCROSSES

Another type of mating gives precise information about a segregating population. This is known as the testcross, a cross of a heterozygous F_1 by the recessive parent. In the F_1 between tall and dwarf peas, or tall and dwarf corn, it is represented by the following:

$$D/d \text{ (tall } F_1) \times d/d$$

The D/d F_1 produces two kinds of gametes, D and d . The homozygous dwarf produces but one kind, d , which combines with two different gametes of the F_1 , giving D/d plants and d/d plants in equal numbers, a 1:1 ratio.

Testcrossing an F_1 individual by the recessive enables the experimenter virtually to look at the gametes of the F_1 individual, since the recessive gamete can in no way mask the appearance of the dominant type, D . In this type of cross the phenotypic and genotypic ratios obtained are identical. The individual formed by the union of two gametes is called a *zygote*. A zygote that is produced by two like gametes is called a *homozygote*, whereas one formed by a union of different gametes is called a *heterozygote*.

A testcross for red hair in humans would be the marriage of an R/r individual by one that is r/r . A family living near Charlottesville, Virginia, represents such a case. There are six children in the family, two non-red and four red. Among the red-haired individuals no two are exactly alike. Two would be classed as auburn, one as brilliant red, and one as golden red, suggesting the presence of modifying genes working on the basic red gene. This difference in shade of red could also be brought about by different alleles at the r locus.

DIFFERENT MONOGENIC CHARACTERS STUDIED BY MENDEL

In using the hybrids of the tall and dwarf peas as an illustration, I have failed to mention the other six characters that Mendel studied. These are listed in Table 3-6.

Plants with gray-brown seed coats also had violet red flowers; those with white seed coats had white flowers. This is what is known as a *pleiotropic* effect, in which the same gene affects different parts of the organism. It is noted that the new term pleiotropic is introduced. Pleiotropy may be defined

Table 3-6. Genes in Peas Studied by Mendel

Part of Plant Affected	Dominant Type	Recessive Type
(1) Seed shape or form	round	wrinkled
(2) Cotyledon color	yellow	green
(3) Seed coat color	gray-brown	white
(4) Pod form	simply inflated	constricted
(5) Color unripe pod	green	yellow
(6) Distribution flowers	axial	terminal
(7) Plant height	tall	dwarf

as the effect of a single gene upon two or more characters not obviously related, in this case seed coat color and flower color.

Table 3-7 lists the ratios found by Mendel in his classic experiments. He solved the difficulty of determining whether ratios were significant by amassing totals large enough so there was no question about the ratios. He observed that the progenies of single plants often gave wide deviations from the expected ratios. For example, in one experiment a plant produced 43 round and only two wrinkled seeds when a 3:1 ratio was expected. In another experiment there were 32 yellow seeds with but one green seed, another plant with 20 yellow and 19 green, where a 3:1 ratio was expected.

Mendel states, "These two experiments are important for the determination of average ratios because with a smaller number of experimental plants they show that very considerable fluctuations may occur." He was aware of the difficulty of basing genetic concepts upon limited populations, and he solved it by amassing large totals. His data are found in Table 3-7.

Table 3-7. Ratios Found by Mendel in F_2 Segregating Progenies of Crosses in Peas

Exp. No.	Characters Studied	Dominant	Recessive	Total	Ratio
(1)	Seed form	5474 round	1850 wrinkled	7324	2.96:1
(2)	Cotyledon color	6022 yellow	2001 green	8023	3.01:1
(3)	Seed coat color	705 gray-brown	224 white	929	3.15:1
(4)	Pod form	882 inflated	299 restricted	1181	2.95:1
(5)	Color unripe pod	428 green	152 yellow	580	2.82:1
(6)	Flower position	651 axial	207 terminal	858	3.14:1
(7)	Plant height	787 tall	277 dwarf	1064	2.84:1
	Total	14,949	5010	19,959	2.98:1

Mendel observed that the recessive seeds bred true in all cases, which was expected since they were homozygous for the characters studied.

When the dominant seeds were planted, approximately one third of them bred true, while the other two thirds showed a segregation similar to the F_2 . In the first two experiments, the results were as shown in Table 3-8. An almost perfect 1:2 ratio was found, with a deviation of only 2 in each case.

Table 3-8. Progenies Obtained from Planting Seed of Dominant Type A/—

	A/A	A/a	Total
Exp. 1	193	372	565
Exp. 2	166	353	519
Total plants	359	725	1084
Expected 1:2	361	723	
Deviation	2	2	

For some of the other five characters studied the results showed more variation. In these cases Mendel was studying plant characters rather than those affecting the cotyledons. He grew progenies from 100 plants of each dominant type instead of growing all the dominant seed (Table 3-9).

Table 3-9. Progenies Obtained by Planting 100 F₂ Seeds Segregating for Different Characters

Exp. No.	Characters Studied	A/A	A/a	Total Seeds
(3)	Seed coat color	36	64	100
(4)	Pod form	29	71	100
(5)	Color unripe pod	40	60	100
(6)	Flower position	33	67	100
(7)	Plant height	28	72	100
	Total plants	166	334	500
	Expected	167	333	
	Deviation	1	1	

In these figures there was a remarkable coincidence between expected and obtained results. Mendel was somewhat disturbed about the 40:60 ratio, so he repeated this experiment and obtained in the second trial 35:65. He concluded, "The average ratio of 2 to 1 appears, therefore, as fixed with certainty."

It should be noted that Mendel did not have the statistical methods of today to tell him whether his results were significant. Instead he had the extraordinary common sense to repeat an experiment if the accuracy was in doubt. Statistical methods can never be substituted for common sense, but the latter may substitute very nicely on occasion for statistical methods.

MONOGENIC INHERITANCE IN DROSOPHILA

Although the original laws of inheritance were discovered in plants, it has been demonstrated many times that the laws apply equally to animals. The most widely investigated animal is *Drosophila melanogaster*, the fruit fly (sometimes called the vinegar fly), first studied genetically by Thomas Hunt Morgan and his associates at Columbia University early in the 20th century. Castle, then at Harvard University, suggested the fruit fly to Morgan. (After a lifetime of teaching and research Castle is now retired at Berkeley, California, but he is still engaged in genetic studies at the age of 94 years. He

is shown in a recent news picture (Fig. 3-8). A photograph of Morgan and R. A. Emerson is found in Fig. 3-9.

The eye colors, brown *bw/bw* and scarlet *st/st*, have been mentioned before in this chapter. As pointed out previously, both brown and scarlet have hundreds of genes in common with the wild-type fly, which has a dark red eye with a black dot near the center. The brown and scarlet flies each differ from the wild by a single gene pair. Undoubtedly, in each case a mutation



News photo by Jim Mazzuchi,
Berkeley (Calif.) Gazette

FIG. 3-8 W. E. Castle, January, 1961; aged 93 years.



Courtesy of Mrs. Trevor Teale

FIG. 3-9 T. H. Morgan (left) with R. A. Emerson, at 5th International Congress of Genetics, Ithaca, New York, 1932.

has taken place at a particular locus, giving rise to a fly with a phenotype different from the normal fly with a red eye. Both brown and scarlet are recessive to the wild condition, so that the F_1 individuals *bw/+* or *st/+* are indistinguishable from flies that are homozygous for the wild-type (+) alleles.

The parents, F_1 , and F_2 of a cross between brown and wild are shown in Table 3-10.

A similar diagram can be made for a cross between wild type and scarlet. These genes are located in chromosomes 2 and 3, respectively, two *autosomes*, not sex chromosomes. The inheritance is the same whether the mutant is used as the male or female, different from that for characters determined by genes located in the X chromosomes where sex-linked inheritance is found. This will be discussed in Chapter 7.

INHERITANCE OF UNUSUAL HAIR CONFORMATION IN RABBITS

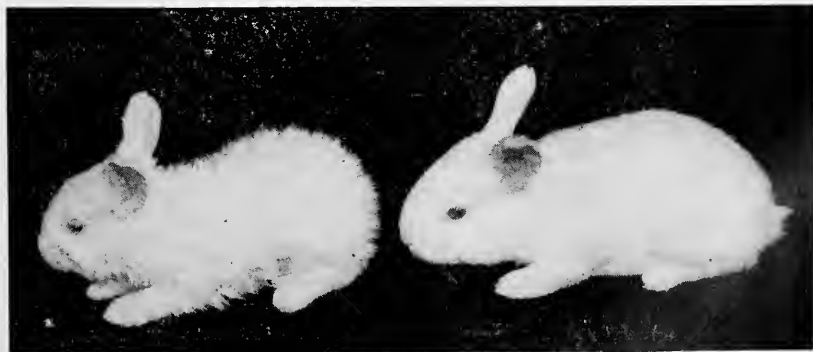
The inheritance of an unusual genetic type has been recently reported in the rabbit (Crary and Sawin, 1959). The mutant "wuzzy" is indis-

Table 3-10. Mating of Red Eye (Wild) \times Brown *Drosophila*

P		
$+/+$ red (wild) eye	(genotype) (phenotype)	bw/bw brown eye
gametes		
$+$		bw
F ₁		
$+/bw$ (red phenotype)		
F ₂		
σ^7 gametes \swarrow ♀ gametes	$+$	bw
$+$	$+/+$ red eye (wild)	$+/bw$ red eye (wild)
bw	$+/bw$ red eye (wild)	bw/bw brown eye

tinguishable from normal until about 10 days old, at which time the hair becomes matted and unkempt (Fig. 3-10). The major effect is one concerning hair morphology, so that the cuticle breaks and matting ensues. Extra secretion from the skin produces a greasy, tacky, or gritty feeling. In addition to entanglement of the hair, there is irritation of the eyes and skin, and secondary localized denudation.

All of these are the pleiotropic effects of a single recessive gene and are



Courtesy of Crary and Sawin; and *Journal of Heredity*

FIG. 3-10 "Wuzzy" rabbit (left) in comparison with normal (right). Note unkempt appearance of wuzzy, a recessive mutation from the normal.

manifest in the homozygous wz/wz genotype. In both F_2 populations and in testcrosses, there is a good fit to a monogenic difference from the normal condition.

YELLOW FAT VERSUS WHITE FAT IN RABBITS

The final example of monogenic inheritance to be cited is one concerning the inheritance of yellow fat in rabbits. In wild rabbits the fat beneath the skin is white, while in certain domestic breeds it is yellow. The gene for white fat is dominant to the one for yellow, which must have arisen by mutation. If we designate the yellow fat y/y , the dominant condition of the wild type would be Y/Y . A cross between a Y/Y genotype and one with y/y would produce an animal with the genotype Y/y , having white fat, because white is dominant. A mating of two F_1 's would produce 3 $Y/-$ (white) to 1 y/y (yellow). (Note the dominant phenotype is written $Y/-$ because it is not possible, except by breeding, to tell whether a dominant individual in the F_2 is Y/Y or Y/y .) The designation $Y/-$ indicates at least one dominant allele, with the dominant phenotype.

This case is particularly interesting because information is available as to the chemical explanation of the yellow pigment. When a wild rabbit eats green plants, in the digestion process the yellow-colored components (*xanthophylls*) are broken down by an enzyme in the liver into colorless derivatives resulting in a white fat. If, however, a mutant rabbit (y/y) lacks the enzyme, the components are not changed and the color of the fat is a distinct yellow.

Apparently the genes act through enzymes, which are organic catalysts. If even one allele for Y is present, the enzyme causes the breakdown of the *xanthophyll* into a colorless compound, resulting in a white fat.

Since one allele of Y apparently is sufficient to cause the destruction of the *xanthophyll*, the addition of another Y allele in the homozygote Y/Y causes an effect indistinguishable from one "dose" of Y in the heterozygote Y/y . It is now generally held that all genes act through enzymes. In most cases one dominant allele will do the job as well as two, giving a plausible explanation of dominance. There are numerous cases of no dominance or cumulative effect. In these there must be quantitative relationship in enzyme production, so that one dose of the allele is less effective than two. Such genes are called cumulative genes.

ORIGIN OF MUTANT CHARACTERS

The distinct hereditary characters we have been studying have all arisen by mutation from a normal or wild stock. In the case of recessive characters, it was necessary for two mutant genes to unite in a zygote before the recessive individual could appear. In animals this would mean that both parents had to be heterozygous for the mutant gene.

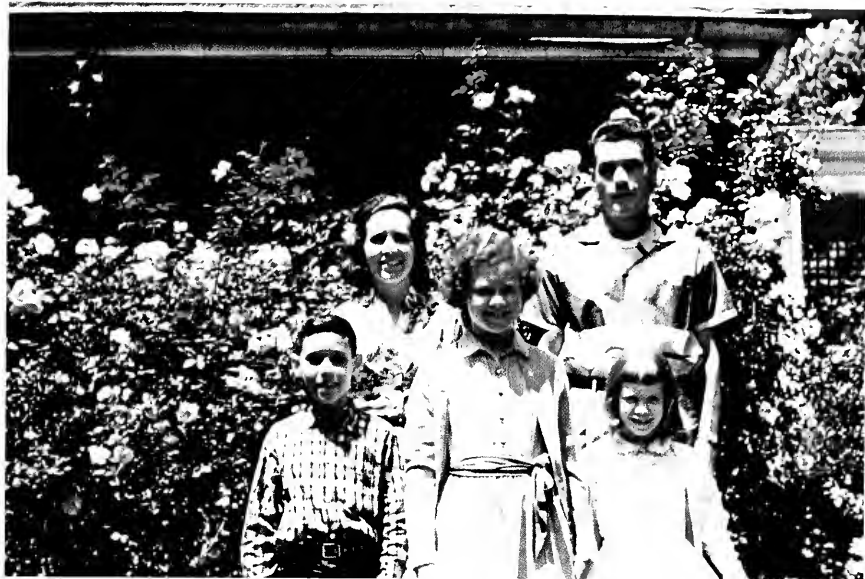


Plate I Segregation for red hair. (*Top*) The mother and father have dark hair R/r while oldest girl has brilliant red hair r/r . Two other children — non-red ($R/-$). (*Bottom*) Family of four girls, all with red hair (r/r). The mother and father both have red hair (r/r). (Courtesy of Fred Ward, Glenview, Illinois.)





Plate II (Top) Chestnut horse (d/d), with full reddish color. (Top right) Palomino horse (D/d), with no dominance. One D allele reduces the color from reddish chestnut color to a beautiful gold. (Bottom right) Cremello horse (D/D), showing cumulative effect of two D alleles. The mating of Chestnut horse with Cremello horse gives 100% palomino.

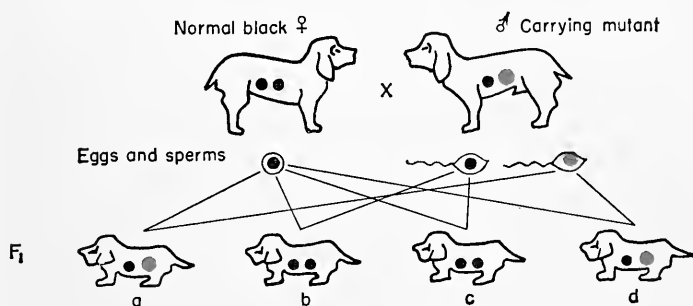
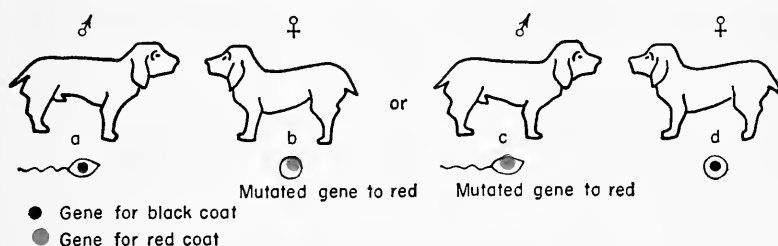




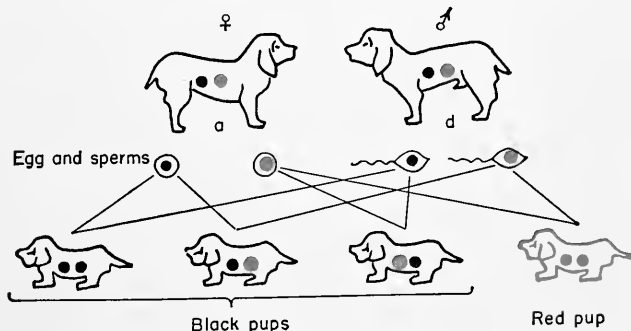
Plate III Four drawings showing eye color in *Drosophila* with the wild, brown, scarlet, and double recessive.

When a dominant gene mutates to a mutant type, there is no other mutant gene with which it can mate, so it unites with a plus allele, forming a heterozygote. In self-pollinated plants, such as peas, one generation is all that is needed for the mutant gene to appear in the population as a homozygous recessive.

In animals, however, the mutant gene may not be rendered homozygous for several generations, or many; it would be carried in the heterozygous condition. If we let m stand for the mutant gene, animals heterozygous for it would be M/m . They would transmit m to one half of their progeny. In the first generation following a mutation, m could mate only with M , producing more



Mating a X c = all black pups
Mating b X c = all black pups
Mating b X d = all black pups



After Charlotte Auerbach; Courtesy of Oliver & Boyd

FIG. 3-11 Origin of a mutant gene in the Cocker Spaniel.

M/m animals. As these became more prevalent in a population there would arise an opportunity for *M/m* animals to mate: when this happened, one fourth of the progeny would be homozygous, with the recessive phenotype, as well as genotype.

This is illustrated in Fig. 3-11, which shows a mutation in the gene for coat color from black to red in either a male or a female Cocker Spaniel. Since mutation is a rare event (only a few in a million), there is little likelihood that two similar mutations would occur in the same individual, or in the same kennel. Hence, the only kind of gene with which the gene for red color could mate is one for black. In Fig. 3-11, the genes for a black coat and for a red coat are indicated by black and red dots, respectively. These are symbols used similarly to gene symbols, for instance *bw* to designate the gene for brown eye in *Drosophila*. The black and red dots (to indicate different genes) simply aid the reader in following the mutant gene (red dot) through the different generations. At last it appears as a homozygous recessive (two red dots).

This mutant gene for red coat color should not be called a "red gene," although this mistake is often made. Actually, a gene is so small that it is not visible with an ordinary light microscope, and no one is certain that it has been seen even with the powerful, newer electron microscope. The gene is an integral part of the chromosome, which has been studied extensively, but mostly in stained material.

In the living organism the chromosome is almost devoid of color. The genes in the chromosomes initiate the action that eventually leads to a distinct phenotypic effect, such as brown eye in *Drosophila* or red coat color in Cocker Spaniels. Genes are not red, or black, or any other color.

CONCLUDING REMARKS

In Chapter 3 we have presented a number of cases of inheritance of unusual phenotypes brought about by single gene mutations from the normal, wild-type condition. In most cases, there is a dominance of the normal condition, so that the homozygous- and heterozygous-dominant individuals are indistinguishable. This is understandable if we assume that the genes act through enzymes and that usually only a very small amount of enzyme is required. Apparently one allele supplies sufficient enzyme to make the wild phenotype, and the amount supplied by a second allele in the homozygous dominant individual is superfluous. In the less frequent cases of no dominance, or cumulative effect, apparently one normal, or "wild," allele does not produce sufficient enzyme to do the job completely; hence the homozygous normal individual (*A/A*) is different from the heterozygous (*A/a*).

The 3:1 ratio found in most material is the result of dominance. With no dominance or cumulative effect, a 1:2:1 ratio is obtained. A good illustration of this is the seedling color in soybeans.

Illustrations were chosen from a wide variety of plants and animals. The same laws of heredity apply to all living things.

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PROBLEMS

3-1. Define the following terms:

cotyledon	Neurospora
dominant	phenotype
dwarf maize	<i>Pisum sativum</i>
element (used by Mendel)	pleiotropic
enzyme	Punnett square
F ₁	recessive
F ₂	self-pollination
gametophytic differences	soybean segregation
genotype	testercross
heterozygote	waxy endosperm
homozygote	waxy pollen
Mendel's law	"wuzzy" rabbit
monogenic	xanthophyll
mutant	yellow fat in rabbits

3-2. Identify the following scientists giving a major contribution of each, with an approximate date.

Beadle, G. W.	Emerson, R. A.
Castle, W. E.	Morgan, T. H.
Dodge, B. O.	Tatum, E. L.

- 3-3. In corn, waxy starch may be observed in the kernel or in the pollen. Normal starch stains blue with iodine and waxy starch, red. In a Wx/wx plant, what per cent of the pollen should stain blue? What per cent red? When selfed, what per cent of the kernels would stain red with iodine?
- 3-4. Show by a Punnett square the different genotypes of kernels expected when a Wx/wx plant is self-pollinated. Write the proper phenotype of each class.
- 3-5. In the preceding problem, the Wx allele is dominant to wx . In fact, one Wx allele is dominant to two wx alleles. The endosperm has 3 n chromosomes, two from the egg and one from the pollen. Modify your Punnett square in Problem 4 to take this into account.
- 3-6. In another endosperm character, floury (fl), the inheritance is also monogenic. However, one gene for normal starch is not dominant to two fl . Two alleles of either kind (Fl or fl) are dominant to one of the opposite kind. Show by a Punnett square the genotypes and phenotypes expected when an Fl/fl plant is self-pollinated.
- 3-7. In *Neurospora* the mycelia (the main part of the plant) are haploid. The only diploid part of the plant is that resulting from fertilization, and before the reduction division at which the haploid number is restored. *Neurospora*, like peas, has a basic chromosome number of 7. How many chromosomes are in the mycelium? How many are in the zygote resulting from fertilization? How many are in the ascospores?
- 3-8. In a strain of *Neurospora* heterozygous for light and dark ascospores, what proportion of light and dark spores results from a single meiosis producing eight ascospores?
- 3-9. In rabbits, the gene Y causes a breakdown of the yellowish xanthophyll plant pigments so that white fat results. In y/y animals an enzyme is lacking so that the xanthophylls are not broken down and yellow fat occurs. How would you feed y/y animals so that the fat produced is white?
- 3-10. In the soybeans reported in this chapter, instead of a 3:1 ratio, a ratio of one green, two light green, and one yellow seedling is obtained. The yellow seedlings soon die. What ratio would be obtained if seed were saved from the green plants? From the light green plants? (Soybeans are self-pollinated.) Write the genotype of all three classes. Which is dominant?
- 3-11. In a corn plant heterozygous for the Wx/wx gene (Fig. 3-4) half of the pollen grains stain blue with iodine (Wx), half red (wx). From a single meiosis four pollen grains would result. Assign a genotype to the four pollen grains along with the proper genotypes of the sperm nuclei resulting from the division of the generative nucleus. The numbers designating the pollen grains are arbitrary. (This problem was suggested by O. J. Eigsti.)

Pollen grains
from 1 meiosis

Genotype of sperm nuclei

No. 1		No. 2	
Wx	wx	Wx	wx

No. 1
No. 2
No. 3
No. 4

Random Distribution of Two or More Genes

IN CHAPTER 3 we were concerned with cases in which the inheritance was conditioned by a single gene pair. In all of these cases the mutant gene, when present in the homozygous recessive individual, a/a , produced a phenotypic effect. With dominance there is a segregation in the F_2 generation of $3 A/- : 1 a/a$. With no dominance the ratio is $1 A/A : 2 A/a : 1 a/a$. It is much like tossing coins. If we toss two pennies enough times, we would expect that in about one fourth of the cases there would be two heads, in one half there would be a head and a tail, and in one fourth, two tails. The probability of a head turning up for any one coin is one half, and likewise the probability for a tail is one half. The probability for two heads in a given throw is $\frac{1}{2} \times \frac{1}{2}$, or $\frac{1}{4}$. The probability holds for tails. There are two ways we can get a head and a tail, so the probability is $2(\frac{1}{2} \times \frac{1}{2}) = \frac{1}{2}$. This is the basic 1:2:1 ratio for the segregation of a single gene pair, with no dominance.

The same law of chance that governs the appearance of heads or tails in coin-tossing applies to the separation of segregating genes when germ cells (gametes) are formed at meiosis in the F_1 , and to the combinations representing the zygotes of the F_2 . Mendel realized this. In fact, his great contribution was the realization that the visible characters observed are due to discrete hereditary determiners. This is sometimes called Mendel's first law.

TWO AND THREE GENE SEGREGATIONS— INDEPENDENT ASSORTMENT

In Mendel's paper there is a section entitled "The Reproductive Cells of the Hybrids," in which he describes what takes place when gametes

are formed. He reasoned that pure strains are reproduced when egg cells and the fertilizing pollen are of like character. He reasoned that exactly similar factors must be at work in the production of constant forms in the hybrid plants, and concluded that, in the ovaries and anthers of the hybrids, as many kinds of germ cells are found as there are possible, constant, recombinations.

We have seen in the hybrids between the dwarf pea and the tall one, and also between tall and dwarf corn, D/d , that there are two possible gametes produced when the germ cells are formed, D and d . If the plant is self-pollinated, the two possible egg types may unite with the two possible sperm in the pollen grains, giving a ratio of 1 D/D :2 D/d :1 d/d . With dominance it is not possible to tell the difference in appearance between plants of the composition D/D and D/d , and a 3:1 ratio is obtained.

In this chapter we are concerned with two or more gene pairs segregating at the same time. We may return to our coin-tossing. If, instead of tossing two pennies, we toss a penny and a nickel at the same time, it is analogous to two genes segregating in one F_1 individual. We know that for one penny the probability of a head's appearing in any given throw is one half. It is the same for the nickel. The probability for a head's appearing in both the nickel and the penny is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$. In other words, we should get the following distribution of heads and tails for a nickel (N) and a penny (P) if we throw these coins enough times:

$$\begin{array}{l} \frac{1}{4} \text{ HP HN} : \frac{1}{4} \text{ HP TN} \\ \frac{1}{4} \text{ TP HN} : \frac{1}{4} \text{ TP TN} \end{array}$$

Since we have two kinds of coins, we can identify all four of these possibilities and know that they are each produced with equal frequency.

We cannot look into the anthers or ovaries of a plant and note the distribution of the different genes at meiosis, but we can infer from the breeding results what took place. This Mendel realized, and from his breeding results he inferred (correctly) that the assortment of the different alleles of two gene pairs is completely at random.

If we assign the letters, A/a and B/b , to two segregating genes, we would expect to obtain the following types of gametes: AB , Ab , aB , and ab —each with the same frequency, or in one fourth of the cases. These four types of gametes represent all of the possible types of segregation for two gene pairs, and they will appear in both the male and female gametes. Each one of these gametes will, by chance, unite with a gamete from the opposite sex, and the frequency will be the product of the probabilities of the individual gametes. The probability for any combination of two genes AB , Ab , aB , and ab , respectively, is one fourth in each case. The probability for a double recessive individual, $a/a b/b$, is $\frac{1}{4} \times \frac{1}{4} = \frac{1}{16}$. In other words, $\frac{1}{16}$ of the F_2 population for two segregating gene pairs will be double recessive $a/a b/b$.

The frequency of *all* the different combinations of gametes to form zygotes can be found by multiplying *all* of the male gametes by *all* of the female gam-

etes. This is most commonly done in a checkerboard by placing the female gametes on one axis and the male gametes on the other. By filling in the squares it is then possible to obtain the genotypes of all 16 F_2 individuals. This is shown in the following conventional checkerboard, or Punnett square (Table 4-1).

Table 4-1. All Possible F_2 Genotypes Produced When F_1 is Heterozygous for Two Gene Pairs A/a and B/b

♂ gametes ♀ gametes	$\frac{1}{4} AB$	$\frac{1}{4} Ab$	$\frac{1}{4} aB$	$\frac{1}{4} ab$
$\frac{1}{4} AB$	$\frac{1}{16} \frac{AB}{AB}$	$\frac{1}{16} \frac{Ab}{AB}$	$\frac{1}{16} \frac{aB}{AB}$	$\frac{1}{16} \frac{ab}{AB}$
$\frac{1}{4} Ab$	$\frac{1}{16} \frac{AB}{Ab}$	$\frac{1}{16} \frac{Ab}{Ab}$	$\frac{1}{16} \frac{aB}{Ab}$	$\frac{1}{16} \frac{ab}{Ab}$
$\frac{1}{4} aB$	$\frac{1}{16} \frac{AB}{aB}$	$\frac{1}{16} \frac{Ab}{aB}$	$\frac{1}{16} \frac{aB}{aB}$	$\frac{1}{16} \frac{ab}{aB}$
$\frac{1}{4} ab$	$\frac{1}{16} \frac{AB}{ab}$	$\frac{1}{16} \frac{Ab}{ab}$	$\frac{1}{16} \frac{aB}{ab}$	$\frac{1}{16} \frac{ab}{ab}$

Summary of phenotypes in Table 4-1 with dominance:

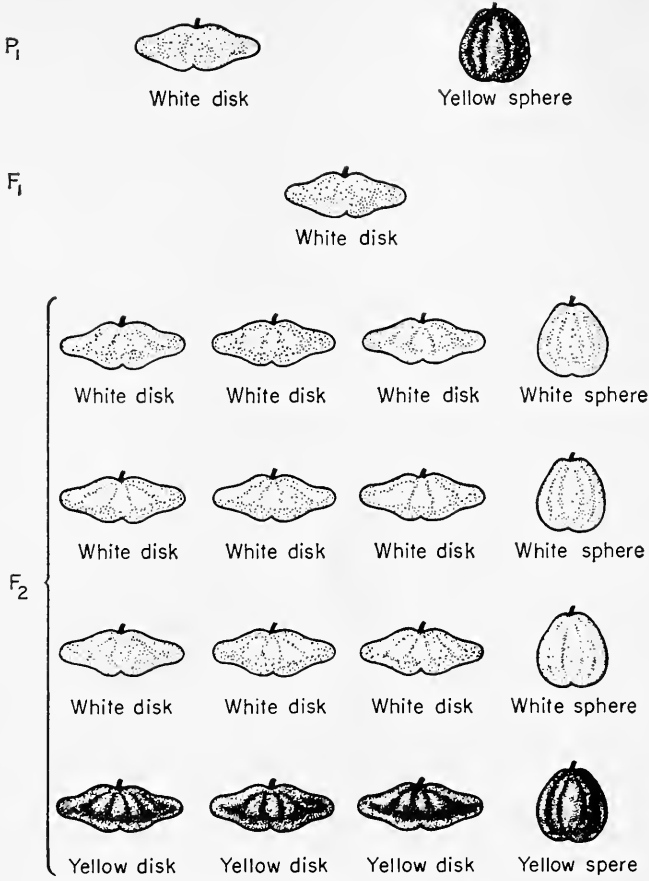
$$\begin{array}{ll}
 \frac{9}{16} A/- & B/- \\
 \frac{3}{16} A/- & b/b \\
 \frac{3}{16} a/a & B/- \\
 \frac{1}{16} a/a & b/b
 \end{array}$$

The segregation of two genes for shape and color of squashes is shown in Fig. 4-1.



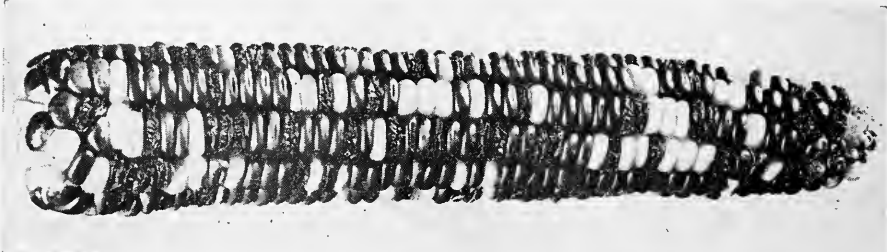
RANDOM ASSORTMENT OF GENES IN GAMETE FORMATION

Independent segregation is found when the genes are distributed to the gametes entirely by chance in a random assortment. Such a distribution occurs when genes are located in different chromosomes. The seven characters in peas studied by Mendel have been shown by later researches to be located in the seven different chromosomes. This is perhaps the only element of luck in Mendel's experiments, that the morphological characters he chose for his investigations all were located in different chromosomes. Had two of them



Courtesy of E. W. Sinnott and K. Wilson; and McGraw-Hill Book Company

FIG. 4-1 Punnett square showing segregation for two genes in squash. Disk is dominant to spherical shape; white is dominant to yellow. A ratio of 9/16; 3/16; 3/16; 1/16 is obtained.



Courtesy of Carolina Biological Supply Co.

FIG. 4-2 Ear of corn, showing 9:3:3:1 segregation for colored starchy, colored sweet, white starchy, and white sweet, respectively.

been in the same chromosome, it is possible he would have found nonrandom assortment of these two characters. (This will be discussed in Chapter 8.)

An ear of corn segregating for two gene pairs, *Su/su* and *R/r*, is shown in Fig. 4-2. There is a 9:3:3:1 ratio for *R/— Su/—*, *R/— su/su*, *r/r Su/—*, and *r/r su/su*.

COMBINING INDIVIDUAL SEGREGATIONS,
ONE AT A TIME

Instead of combining the segregations of two genes simultaneously, we may take them one at a time, provided, of course, that they are segregating at random. If we put the segregation for *A/a* on one axis and the

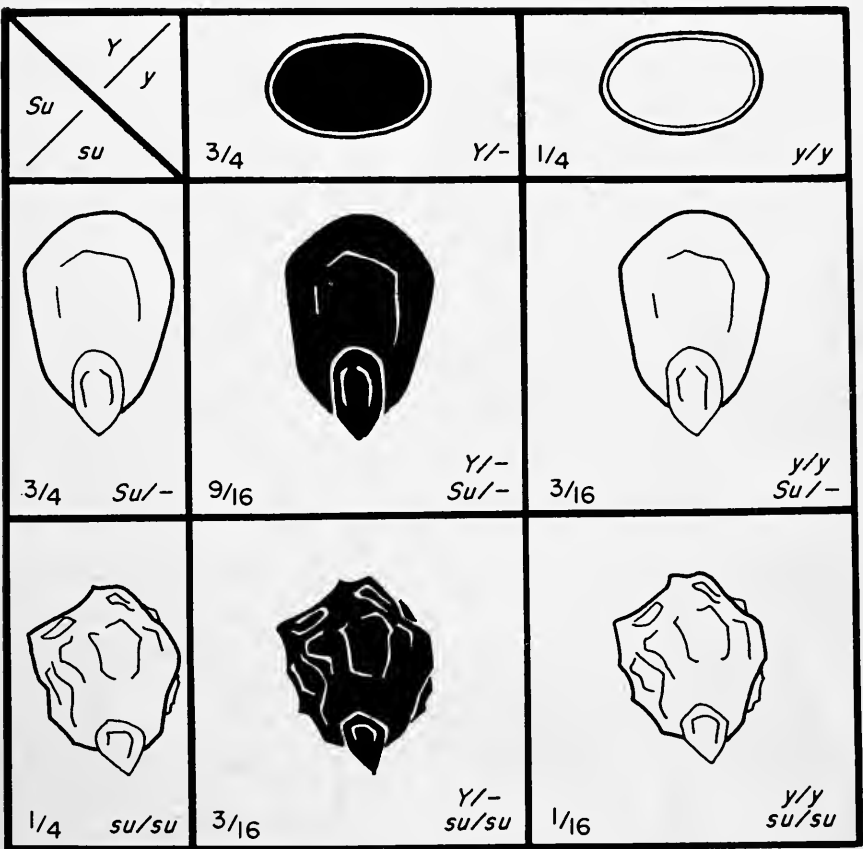


FIG. 4-3 Zygotic checkerboard illustrating segregation of yellow/white endosperm (horizontal axis) and starchy/sugary endosperm (vertical axis). The proportions of different phenotypes can be observed directly.

Table 4-2. F₂ Segregation for Genes A/a and B/b Taken Separately (Zygotic Checkerboard)

B/b segregation \ Segregation for A/a	$\frac{3}{4}$ A/—	$\frac{1}{4}$ a/a
	$\frac{9}{16}$ A/— B/—	$\frac{3}{16}$ a/a B/—
$\frac{3}{4}$ B/—		
$\frac{1}{4}$ b/b	$\frac{3}{16}$ A/— b/b	$\frac{1}{16}$ a/a b/b

segregation for B/b on the other axis, we get a distribution as shown in Table 4-2. Dominance is assumed.

This zygotic checkerboard is just as logical as the conventional one in which the gametes are listed on the different axes and then are multiplied to

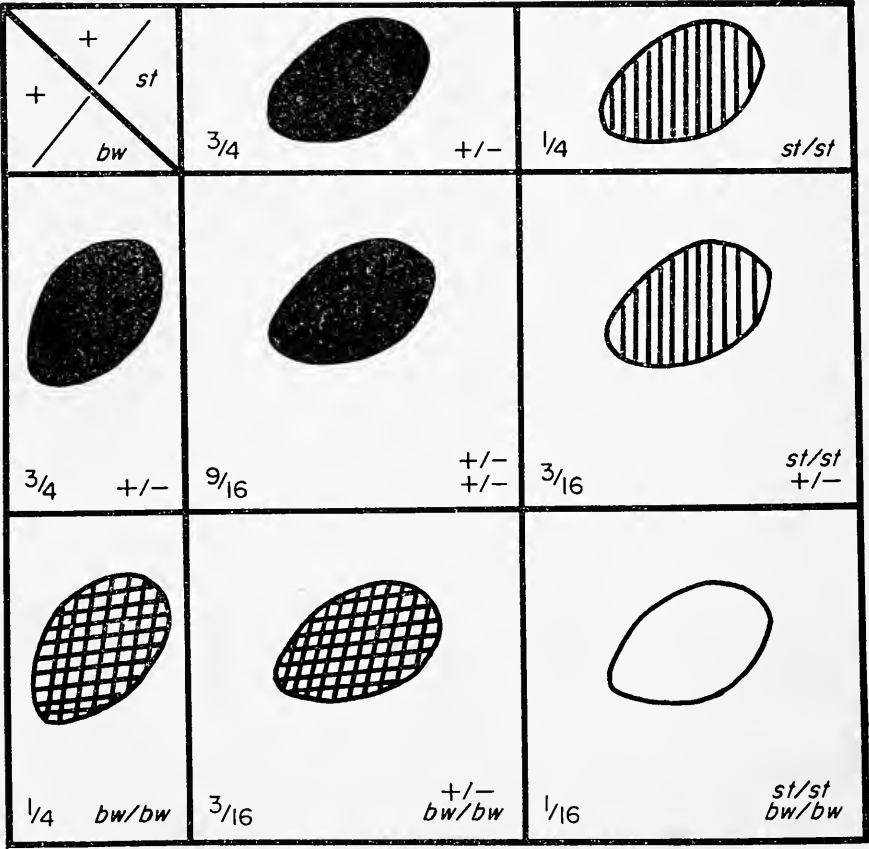


FIG. 4-4 Zygotic checkerboard showing F₂ segregation of brown and scarlet eye color in *Drosophila*. The double recessive is a white eye.

obtain all possible phenotypes. Both methods assume independent assortment of the two genes involved. The zygotic checkerboard showing the segregation for two genes in maize, those for yellow versus white endosperm and for starchy versus sweet kernels, is shown in Fig. 4-3. They are comparable to those governing seed form and color in peas, studied by Mendel.

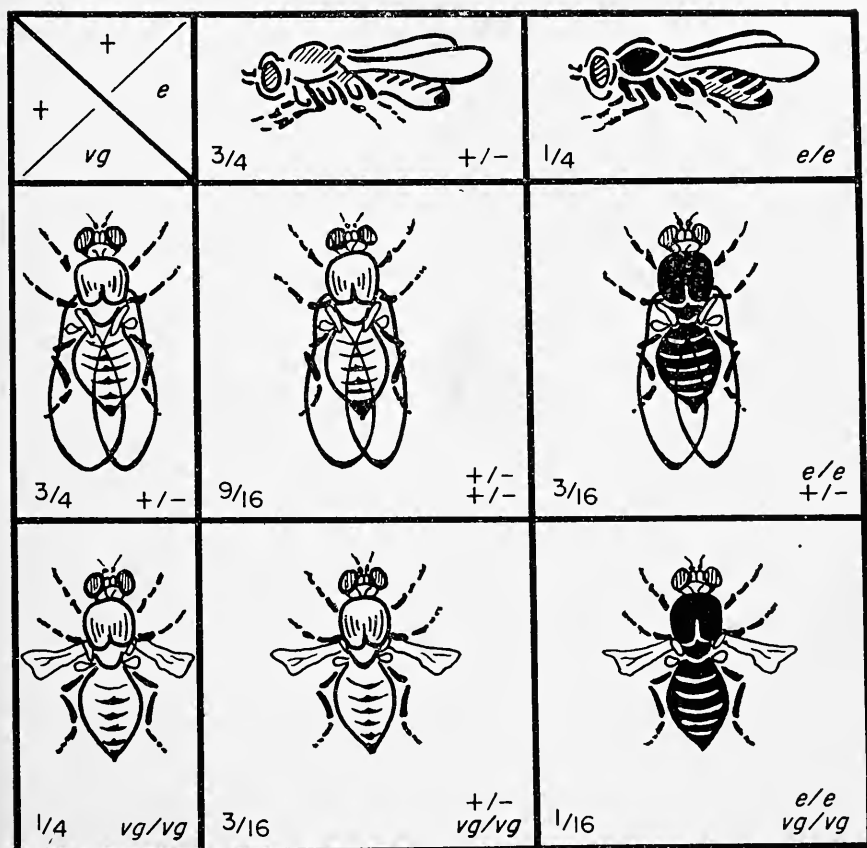


FIG. 4-5 F_2 segregation for normal body color of *Drosophila* versus ebony body, in combination with segregation for normal and vestigial wings. The proportions of different phenotypes are shown.

Zygotic checkerboards showing the segregation of brown and scarlet in *Drosophila* and also the segregation of ebony and vestigial are shown in Figs. 4-4 and 4-5. The F_2 segregation for a cross between a brown and scarlet is also illustrated in Plate III. The striking thing about this segregation is that the double recessive, *bw/bw st/st*, is white, with no pigment.

If it is desired to determine the phenotypes when three genes are segregating, it can be done indirectly in a two-step process. One can first determine the

phenotypes when two genes are segregating and then place these phenotypes on one axis, with the phenotypes for the third gene on the other axis. This is shown in Table 4-3. Dominance is assumed in each case.

Table 4-3. Zygotic Checkerboard Showing Segregation of Three Genes A/a , B/b , and C/c

Segregation for A/a B/b C/c	$\frac{9}{16}$ $A/-$ $B/-$		$\frac{3}{16}$ $A/-$ b/b		$\frac{3}{16}$ a/a $B/-$		$\frac{1}{16}$ a/a b/b	
$\frac{3}{4} C/-$	$A/-$ $B/-$ $C/-$	$\frac{27}{64}$	$A/-$ b/b $C/-$	$\frac{9}{64}$	a/a $B/-$ $C/-$	$\frac{9}{64}$	a/a b/b $C/-$	$\frac{3}{64}$
$\frac{1}{4} c/c$	$A/-$ $B/-$ c/c	$\frac{9}{64}$	$A/-$ b/b c/c	$\frac{3}{64}$	a/a $B/-$ c/c	$\frac{3}{64}$	a/a b/b c/c	$\frac{1}{64}$

INHERITANCE OF OBESITY IN MICE

An interesting example of an unusual condition, the result of a single gene differences from the normal, is obesity in mice. The obese animals are about twice the weight of normal mice, which is due to a recessive gene. The first case was described in 1950, and the gene symbol *ob* was given for obese. Recessive animals *ob/ob* show this characteristic, while *Ob/Ob* and *Ob/ob* are normal, since *Ob* is dominant. The *ob/ob* animals also are sterile.

A second gene, causing a similar phenotype, has been observed recently (1959) and given the gene symbol *ad* for adipose. It is recessive. A picture of *ad/ad* animals in comparison with the normal is shown in Fig. 4-6.

Segregation for the gene *ad* is good, giving about 25% adipose in F_2 progenies. Matings of *Ad/ad* animals with *Ob/ob* heterozygotes have always resulted in normal individuals, which shows that the two genes are not allelic. It is not possible to mate *ob/ob* animals with *ad/ad* since both recessives are sterile.

A double homozygote (*ad/ad dw/dw*) for adipose and for pituitary dwarf is shown in Fig. 4-6, with phenotypes in an F_2 segregating for adipose and dwarf. Apparently the dwarfism does not interfere with the adiposity, nor does the adiposity affect the skeletal growth of the dwarf.

The genes *ob* and *ad*, which cause excessive fat production in mice, represent extreme cases of the action of single genes. The heredity has been well established in a good experimental animal, the mouse. Whether obesity in



Courtesy of D. S. Falconer; and Journal of Heredity

FIG. 4-6 F_2 segregation for adipose (ad/ad) and dwarf (dw/dw). Four phenotypes from left to right are $Ad/- Dw/-$, $ad/ad Dw/-$, $Ad/- dw/dw$, and $ad/ad dw/dw$. Ratio expected is 9:3:3:1.

man may be due to a single gene is not certain, but there are undoubtedly genetic differences for excess weight in man.

THE BRACKET METHOD OF DESIGNATING MULTIPLE GENE SEGREGATION

A third way of designating segregations for more than one gene is known as the bracket or branching method (Table 4-4), a relatively old one for taking one gene pair at a time, as is done in the zygotic checkerboard.

Where more than two genes are segregating, the bracket method is perhaps more useful than the zygotic checkerboard. Either method is preferable to the old-fashioned checkerboard, where all the gametic combinations are written on both axes and the zygotes produced by all possible unions of the different gametes are seen in the resulting squares.

Students of course will realize that these methods are but mechanical aids in ascertaining all the possible phenotypes in a segregating population. The method easiest for the individual student should be employed. Time spent on the mechanics of segregation should be minimized so that the student will be able to concentrate on an understanding of the biological processes underlying gene action.

Table 4-4. Bracket Method for Combining Segregations of Individual Genes (Dominance Assumed)

Gene A/a	Gene B/b	Combination A/a and B/b	Combination A/a B/b and C/c
$\frac{3}{4}$ A/—	$\left\{ \begin{array}{l} \frac{3}{4} B/— \\ \frac{1}{4} b/b \end{array} \right.$	$\frac{9}{16}$ A/— B/—	$\left\{ \begin{array}{l} \frac{27}{64} A/— B/— C/— \\ \frac{9}{64} A/— B/— c/c \end{array} \right.$
		$\frac{3}{16}$ A/— b/b	$\left\{ \begin{array}{l} \frac{9}{64} A/— b/b C/— \\ \frac{3}{64} A/— b/b c/c \end{array} \right.$
$\frac{1}{4}$ a/a	$\left\{ \begin{array}{l} \frac{3}{4} B/— \\ \frac{1}{4} b/b \end{array} \right.$	$\frac{3}{16}$ a/a B/—	$\left\{ \begin{array}{l} \frac{9}{64} a/a B/— C/— \\ \frac{3}{64} a/a B/— c/c \end{array} \right.$
		$\frac{1}{16}$ a/a b/b	$\left\{ \begin{array}{l} \frac{3}{64} a/a b/b C/— \\ \frac{1}{64} a/a b/b c/c \end{array} \right.$

GENOTYPES IN SEGREGATING PROGENIES

Dominance is assumed in using the three different methods for determining phenotypes when two or more genes are segregating. In the conventional gametic checkerboard, the genotypes can be ascertained, although it is somewhat laborious to pick them out of the checkerboard. The genotypes can also be ascertained by use of the bracket method or the zygotic checkerboard, if the dominant classes in each case are separated into homozygotes and heterozygotes. Such a segregation may be seen in Table 4-5. Thus for two segregating genes there are nine different genotypes. It is much easier to see these directly than to pick them out of the conventional gametic checkerboard.

NUMBER OF GENOTYPES AND PHENOTYPES WITH N GENES SEGREGATING

It is often desirable to know how many phenotypes and genotypes can be obtained from the segregation of any known number of genes, and the minimum population that will permit the investigator to have an opportunity to obtain the recombinations of all genes present. This information for one to six segregating genes is shown in Table 4-6, as well as the formula for calculating them for any number of genes. It may be seen in this table that the number of phenotypes (2^n) is always the same as the number of gametes. This is a consequence of dominance.

If there is no dominance, there will be as many phenotypes as genotypes. The number of genotypes is greater (3^n), while the population size is greatest of all (4^n). This increases extremely rapidly. We once calculated the size of a corn field that would be necessary to have an opportunity to obtain all zygotes

Table 4-5. Genotypic Distribution for Two Heterozygous Genes

<div>Segregation for A/a</div> <div>B/b segregation</div>	$\frac{1}{4}$ A/A	$\frac{1}{2}$ A/a	$\frac{1}{4}$ a/a
$\frac{1}{4}$ B/B	$\frac{1}{16}$ A/A B/B	$\frac{1}{8}$ A/a B/B	$\frac{1}{16}$ a/a B/B
$\frac{1}{2}$ B/b	$\frac{1}{8}$ A/A B/b	$\frac{1}{4}$ A/a B/b	$\frac{1}{8}$ a/a B/b
$\frac{1}{4}$ b/b	$\frac{1}{16}$ A/A b/b	$\frac{1}{8}$ A/a b/b	$\frac{1}{16}$ a/a b/b

when 30 genes were segregating. Such a corn field would be fantastic, 2000 times the total land area of the earth.

Table 4-6. Data Concerning Genic Constitution and Minimum Population Size for 1—n Genes Segregating (Dominance Assumed)

No. Genes	Kinds of Gametes	No. Phenotypes	No. Genotypes	Population Size ^a
1	2	2	3	4
2	4	4	9	16
3	8	8	27	64
4	16	16	81	256
5	32	32	243	1024
6	64	64	729	4096
n	2 ⁿ	2 ⁿ	3 ⁿ	4 ⁿ

^a This is the minimum population size that would permit the recombination of all genes present.

TESTCROSS INFORMATION

A *testcross* may be defined as a cross of the F_1 by the multiple recessive stock for all segregating genes. In "dihybrids" such as we have been discussing, a testcross would represent the cross of the F_1 by the double recessive. If the F_1 were $A/a B/b$ the testcross would then be $A/a B/b \times a/a b/b$.

Much useful information can be obtained by use of the testcross. For example, in the one above there should be four kinds of gametes formed by the F_1 — AB , Ab , aB , and ab . By the use of the testcross it can be ascertained directly whether these are produced in equal numbers. These gametes each will be united with a gamete ab which can in no way alter the phenotypic expression of any of the progeny. Actually, then, a testcross enables the investigator to determine directly the genotypes of the gametes produced by the F_1 hybrid. This is most important in determining linkage relationships, as will be pointed out in Chapter 8. The number of kinds of individuals produced by a testcross is always the same as the number of kinds of gametes, 2^n , where n represents the number of gene pairs in a heterozygote.

GENE SYMBOLS

It has become customary to assign gene symbols to all mutant types in any organism in which genetics is being studied. This is necessary for the researcher making crosses with many genetic stocks. The customary procedure is to label the mutant type, using an abbreviation for the gene symbol. For example, to Mendel's peas we could assign the term dwarf to describe the short pea plants. The abbreviation for dwarf is d , which is the gene symbol for the recessive. There are different ways of designating the dominants. The corresponding capital letter is often used. Maize geneticists commonly use a plus (+) to designate the dominant type. Drosophila workers may use this system or the gene symbol of the recessive, with a super plus on the symbol for the mutant. For example, white eye = w and the wild-type eye = + or w^+ . This works as well for a dominant mutant as for one that is recessive. For example, the Bar condition in Drosophila is designated B , denoting domi-

Table 4-7. Maize and Drosophila Designations of Recessive and Dominant Alleles

Maize	Recessive	Dominant
Glossy seedlings	gl	Gl or +
Liguleless seedlings	lg	Lg or +
Sweet endosperm	su	Su or +
White endosperm	y	Y or +
Drosophila	Recessive	Dominant
Yellow body	y	y^+ or +
Scarlet eye	st	st^+ or +
Brown eye	bw	bw^+ or +

nance, and the wild-type condition is B^+ . Actually the Bar eye has no dominance. The F_1 is almost exactly intermediate between the two parents, showing a cumulative effect of the number of alleles for Bar.

In many instances, it is not possible to use only one letter to designate mutant types—there are not enough letters in the alphabet. Combinations of two letters are frequently made. Some common ones in maize are listed in Table 4-7.

CONCLUDING REMARKS

This chapter is concerned with the random distribution of two genes in segregating populations. Phenotypes and genotypes are usually obtained by use of the conventional checkerboard, or Punnett square, whereby all possible gametes are placed on both axes of a checkerboard and the progeny obtained by filling in the squares of the checkerboard.

For genes located in different chromosomes, the segregation for each will be independent of segregation for other genes. Consequently, it is logical to write the segregation for one gene on one axis of the checkerboard and the segregation of the second gene on the other axis. This is a zygotic checkerboard.

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PROBLEMS

4-1. Define the following terms:

anther	obese mice
Bar gene	ovary
bracket method	random distribution
branching method	scarlet eye
brown eye	sugary endosperm
gametes	testcross
gene symbol	white endosperm corn
glossy seedling	wild-type eye
independent assortment	yellow body
liguleless seedling	zygotic checkerboard

- 4-2. Identify the following scientist, giving a major contribution with an approximate date.

Punnett, R. C.

- 4-3. In corn the gene for a green plant is dominant to the one for yellow green, and the gene for liguled seedlings is dominant to the one for liguleless. The genes for these two characters are located in different chromosomes. Make a cross between a yellow-green plant and a liguleless plant. Write the genotypes for the parents and the F_1 ; also give the phenotypes of each.
- 4-4. Show by the bracket method the different gametes from the F_1 in Problem 3. By combining these gametes in a Punnett square, show the different genotypes expected. Give the phenotypes for all the genotypes, and show in a summary table how many of each phenotype is expected. What is the ratio?
- 4-5. Combine the segregation for these two genes of corn, one at a time, for phenotypes only, in a zygotic checkerboard. Give the proportion of phenotypes expected.
- 4-6. Make the calculation for the same genes using the bracket method. Show the segregation one gene at a time for phenotypes only.
- 4-7. Calculate the proportion of expected *genotypes* showing the segregation one gene at a time by either the bracket method or the zygotic checkerboard.
- 4-8. In an F_2 population of 800 individuals, how many of each genotype would be expected?
- 4-9. If the F_1 plant were pollinated by a double recessive, how many of each phenotype would be obtained in a population of 1000 plants? How many of the different genotypes would there be? How do the phenotypes and genotypes differ? What is this kind of cross called? What is its advantage in studying inheritance?
- 4-10. Suppose the stocks used in the preceding problems differed in another character, the color of the seed. One had anthocyanin color (A/A); the other was colorless (a/a). Write the genotypes for the two stocks and the F_1 , giving phenotypes for each. The gene A is in a chromosome different from those containing yg and lg . Choose any method you prefer for showing the phenotypes expected in an F_2 population of 1280.

Test of Significance: Chi-Square

IN CHAPTER 3, Mendel's results were cited. While his total results were very close to the expected ratios, Mendel observed that the results of some of the individual F_1 plants showed considerable variation. In one ratio of 40:60 where he expected 33:67, he repeated the experiment and obtained a 35:65 ratio, as was mentioned in Chapter 3. It is highly commendable to repeat an experiment if the result is doubtful, but this is not always possible. Furthermore, the investigator would like to know immediately what the chances are that his results, based on a small sample, represent a true sample of an infinitely larger population.

The statisticians have worked out a formula for supplying this type of information: the chi-square test. This formula takes into consideration the size of the population studied, and it can be illustrated by a testcross population of yellow and white endosperm in corn. The genetic symbols are Y for yellow and y for white. An F_1 with the genotype Y/y , when testcrossed, would give the results shown in Table 5-1.

In two such testcross populations of different sizes, suppose that a deviation of 10 from the expected ratio were found. In a population of 100 seeds, a deviation of 10 from the expected 1:1 ratio would result in 40:60. Would this be considered a significant deviation from a 1:1 ratio? A large population of 1000 seeds with the same numerical deviation of 10 would give a population 490:510, whereas equal numbers of each were expected, or 500:500. Would this be considered a significant deviation from the expected 1:1 ratio? In a third testcross population of 1000 seeds, suppose the *same proportion* of seeds of the two types as obtained in population No. 1 were found, i.e., 400:600. Would this be considered a significant departure from the expected 1:1 ratio?

The chi-square test provides information about these three populations and

Table 5-1. Result of Testcrossing Y/y F_1 Plant (Yellow Seeds) by y/y

F_1 Y/y (yellow seeds)	
$\begin{array}{c} \nearrow \\ \text{♀ gametes} \end{array}$	$\begin{array}{c} \nwarrow \\ \text{♂ gametes} \end{array}$
Y	y (from homozygous y/y)
y	
	Y/y yellow
	y/y white

whether the differences encountered are significantly different from a 1:1 ratio. The formula for chi-square is as follows:

$$\text{Chi-square } \chi^2 = \sum \left(\frac{d^2}{e} \right)$$

In this formula, d represents the deviation from the expected ratio, e represents the expected and Σ is used to denote summation. Table 5-2 shows the chi-squares for the three hypothetical populations.

Table 5-2. Three Testcross Populations Segregating for Yellow and White Endosperm in Corn

	POPULATION SIZE					
	No. 1 100		No. 2 1000		No. 3 1000	
	yellow (Y)	white (y)	yellow (Y)	white (y)	yellow (Y)	white (y)
Observed	40	60	510	490	400	600
Expected (e)	50	50	500	500	500	500
Deviation (d)	10	10	10	10	100	100
d^2	100	100	100	100	10,000	10,000
d^2/e	2	2	0.2	0.2	20	20
$\chi^2 \Sigma \left(\frac{d^2}{e} \right)$	4		0.4		40	

The smaller the chi-square, the more likely it seems that only chance is operating to give a deviation as large as the one encountered. Thus it is seen that the second population with a chi-square of only .4 agrees most closely with the expected ratio. The first population of 100 seeds, while showing

numerically the same deviation as population No. 2, has a much larger chi-square. There would be more cause to doubt the fact that this represented a 1:1 segregation. In the case of the third population, it would seem extremely unlikely that this actually represented a 1:1 segregation. There was the same proportion of yellow and white seeds as in population No. 1. However, the fact that so many more seeds entered into the calculation gives it an entirely different chi-square. The investigator encountering such results might well look for some explanation, other than chance fluctuation, to explain such a wide deviation.

This hypothetical table shows that, with a large population, a deviation *numerically* the same as the smaller population (No. 2 as compared with No. 1) can be tolerated more readily than the same *numerical* deviation in the small one. Compare the chi-square of .4 against that of 4. The latter is ten times as great.

However, if the deviation in the larger population is proportionally the same as the smaller (No. 3 as compared with No. 1), it is more likely that the deviation in the larger one is statistically different from the expected. The chi-square of 40 for population No. 3 makes it seem almost certain that the deviation from the expected ratio is significant.

PROBABILITY

The statisticians have also developed methods for estimating the probability that any observed chi-square represents a chance fluctuation. Most of the probabilities encountered range from .001 to .999, with the former representing a very low probability and the latter a very high one that the results found agree with the expected. Probability values for any chi-square may be determined from Table 5-3. These values range from .99 to .001. Let us consider the probability value of the first column, .99. This can be interpreted to mean that in 100 trials, 99 times we would expect to find as large a deviation from the expected ratio. In other words, the deviation from the expected ratio is very low.

For comparison let us look at the P value of .001 at the opposite side of the table. Here the deviation from the expected, as shown by a high chi-square, is rather large. The P value of .001 tells us that we would expect to find a deviation as large as this only once in 1000 trials, due to chance alone. When an investigator encounters such a result, he is confident that something other than chance fluctuation is influencing the result. Even a probability of .01 signifies that it is likely that something other than chance is operating. This is what is commonly known as a significant deviation, sometimes called "significant at the .01 level."

Likewise, when we encounter a P value of .05 (third from the last column) we would expect such a deviation, by chance alone, from an infinitely large population only once in 20 times. Most research workers consider a P

value of .05 a good indication that something other than chance fluctuation is operating. This is considered barely significant, and sometimes may be called "significant at the .05 level."

DEGREES OF FREEDOM

Degrees of freedom is a term used to show how many variables are operating in the test under consideration. This must be taken into account when determining whether the chi-square indicates a significant deviation from the expected ratio. The number of degrees of freedom is almost always one less than the number of classes. For example, with two classes, such as the yellow and the white endosperm of corn, there is only one degree of freedom. If there were only two kernels of corn in a bag, one yellow and one white, there would be only one degree of freedom in taking these out of the bag in order. You may select either of the kernels on the first trial—this is the one degree of freedom. Once a yellow or a white kernel has been chosen, there is no choice in the drawing of the second. The remaining one must be second. In a 1:1 segregation or a 3:1 segregation, there is but one degree of freedom. In a 1:2:1 segregation there are two degrees of freedom, because there are three classes. When we encounter more classes, there will be more degrees of freedom, one less than the number of classes under observation.

IMPROPER USE OF CHI-SQUARE

There are two reservations regarding the use of chi-square in determining significance.

1. Chi-square must be determined on the numbers themselves and not on percentages or ratios of the number. Since one of the factors, a primary one, in determining any chi-square is the size of the population, we must not reduce (or increase in some cases) every population to a basis of 100 before making the calculations.
2. Chi-square cannot properly be used for distributions in which any class is less than 5, and most statisticians prefer a larger figure such as 50 for the minimum number.

By using Table 5-3 it is possible to determine the probabilities of the three hypothetical populations in Table 5-2. Since there are two types of kernels in each case, there is one degree of freedom. Consequently, the chi-squares for the three populations are found in the top row of figures for chi-squares.

The chi-square for population No. 1 is 4. The probability for such a value (P) is slightly less than .05. The chi-square for .05 is actually 3.8.

For the second population with a chi-square of .4 we see that the P value is slightly greater than .50. Hence we would expect a deviation as large as the

Table 5-3. Distribution of χ^2 Probability, Abridged^a

Degrees of Freedom	Probability								
	0.99	0.90	0.70	0.50	0.30	0.10	0.05	0.01	0.001
	Chi-Square Values								
1	0.0002	0.016	0.15	0.46	1.1	2.7	3.8	6.6	10.8
2	0.02	0.21	0.71	1.39	2.4	4.6	6.0	9.2	13.8
3	0.12	0.58	1.42	2.37	3.7	6.3	7.8	11.3	16.3
4	0.30	1.06	2.20	3.36	4.9	7.8	9.5	13.3	18.5
5	0.55	1.61	3.00	4.35	6.1	9.2	11.1	15.1	20.5
6	0.87	2.20	3.83	5.35	7.2	10.6	12.6	16.8	22.5
7	1.24	2.83	4.67	6.35	8.4	12.0	14.1	18.5	24.3
8	1.65	3.49	5.53	7.34	9.5	13.4	15.5	20.1	26.1
9	2.09	4.17	6.39	8.34	10.6	14.7	16.9	21.7	27.9
10	2.56	4.87	7.27	9.34	11.8	16.0	18.3	23.2	29.6

^a Courtesy of Fisher and Yates, Oliver and Boyd Ltd., Edinburgh, and Hafner Publishing Company, New York.

one observed in approximately one half of a large number of such trials. In other words, the observed result does not differ significantly from the expected.

The third population presents an entirely different picture. The chi-square of 40 is so large it is not found in Table 5-3. Even a chi-square of 10.8 (the highest given for one degree of freedom) gives the probability .001. Hence the P value for population No. 3 is $< .001$. It is even less than .0001 (from another table), which would mean that a deviation as great as the one encountered would be expected less than once in 10,000 trials. It is almost certain that some factor, other than chance, is operating to produce population No. 3. If we were tossing a coin 1000 times and got 600 heads and 400 tails, we would be justified in suspecting that the coin was loaded in some way to make the appearance of heads more frequent.

There are genetic factors that cause distortion of ratios similar to that in population No. 3. If an investigator found such a distorted ratio he would design experiments to determine the cause.

EXPERIMENTAL USE OF CHI-SQUARE

Every investigator, when counting ratios of segregating progenies, is sometimes in doubt as to whether his experimental results agree with the expected ratio. Perhaps it would be well to examine data from a few experiments, apply the chi-square test, and determine the probability of agreement with an expected ratio. For examples we shall use some of the ratios first counted in corn in the classic studies of East and Hayes (1911). This work was done at the Connecticut Agricultural Experiment Station, the first laboratory of its kind in this country.

Let us take the segregation for starchy (*Su*) and sugary (*su*) endosperm in corn. These characters are analogous to the ones studied in peas by Mendel. Table 5-4 shows three families consisting of 26 ears, all self-pollinated.

Table 5-4. Chi-square Determination for Three Families of Corn Segregating for *Su/su* (data from East and Hayes)

	Family 1		Family 2		Family 3	
	starchy (<i>Su</i>)	sweet (<i>su</i>)	starchy (<i>Su</i>)	sweet (<i>su</i>)	starchy (<i>Su</i>)	sweet (<i>su</i>)
Observed	1505	530	3524	1163	2190	725
Expected (e)	1527	509	3516	1172	2187	729
Deviation (d)	22	21	8	9	3	4
d ²	484	441	64	81	9	16
d ² /e	0.3	0.9	0.02	0.07	0.004	0.022
$\chi^2 = \sum d^2/e$	1.2		0.09		0.026	

The chi-square values for the three families were 1.2, .09, and .026. These are all low values. By referring to Table 5-3, the probabilities (P) for these three different chi-squares can be determined. For a chi-square of 1.2, with one degree of freedom, we see the probability (P) is slightly less than .30. This means that in 100 such trials, approximately 30 would be expected to show a deviation from a 3:1 ratio as great. The P value for the other two values is approximately .90, a practical certainty that the ratios obtained actually represented 3:1 segregations. This is not surprising, since all of the deviations from a 3:1 ratio were not large, even for family 1. In these cases the application of the chi-square test was hardly necessary. It is most useful to have this test for doubtful cases.

CONCLUDING REMARKS

This chapter is concerned with a statistical method, the chi-square test. [This method is used for estimating whether experimental ratios differ significantly from the expected proportion (based on random distribution of the genes). It is of utmost importance to use the chi-square only in cases where it applies, and to substantiate conclusions with experimental data in doubtful cases. Calculations were made for three sets of experimental data.

REFERENCES

- East, E. M., and H. K. Hayes. 1911. Inheritance in maize. 142 pp. *Conn. Agr. Exp. Sta. Bull.* 167.
- Fisher, R. A., and Frank Yates. 1949. *Statistical Tables for Biological, Agricul-*

tural and Medical Research. 112 pp. Oliver and Boyd, Ltd., Edinburgh, and Hafner Publishing Co., New York.

PROBLEMS

5-1. Define the following terms:

chi-square	expected ratio
degrees of freedom	probability
deviation	

5-2. Identify the following scientists giving a major contribution of each, with an approximate date.

East, E. M.
Hayes, H. K.

5-3 to

5-9. Using Table 3-7 of Chapter 3, calculate the chi-square and the probability (P) for all the seven characters studied by Mendel.

5-10. Calculate the chi-square and probability for both experiments 1 and 2, and also for a combination of these two experiments of Mendel, in Table 3-8.

Influence of Environment on Heredity

IN THE preceding chapters the various characters studied were considered independent of the environment. The genes responsible were assumed to produce their effects regardless of the cultural conditions surrounding the individual plant or animal. This, of course, is an oversimplification of the case. Some genes do produce a rather consistent effect even in diverse environments. For example, the tall peas studied by Mendel or the tall corn produce plants much taller than their dwarf counterparts irrespective of fertility of the soil, or whether the temperature is cool or warm.

Other characters respond differently. Robertson and Anderson (1961) reported two seedling mutants in maize—pastel and white-mutable—alleles of the y_1 locus, that are temperature-sensitive. If grown in a warm temperature, $37^\circ\text{C}.$, plants homozygous for these alleles are pale green as seedlings and as mature plants. If grown under low temperature, $20^\circ\text{C}.$, it is difficult to distinguish the mutants from normal.

This is in marked contrast to virescent seedlings in maize, whose expression is more pronounced in cool temperatures. In the spring with low temperature, the virescents are clearly visible as yellowish green seedlings. They turn green after a few days. Afterward it is impossible to distinguish them from the green plants (V/V , or V/v).

However, if the virescent seedlings are grown in the early summer, when the temperature is much warmer, they either remain yellowish green for a very short time or emerge as fully green seedlings, although the genotype is v/v (Fig. 6-1). In this case there is an interaction between the heredity and the environment. In the early days of genetics controversies arose as to whether heredity or environment was more important. Actually both are important. The heredity determines the potential and the environment determines to what extent the individual reaches its potential. This may be

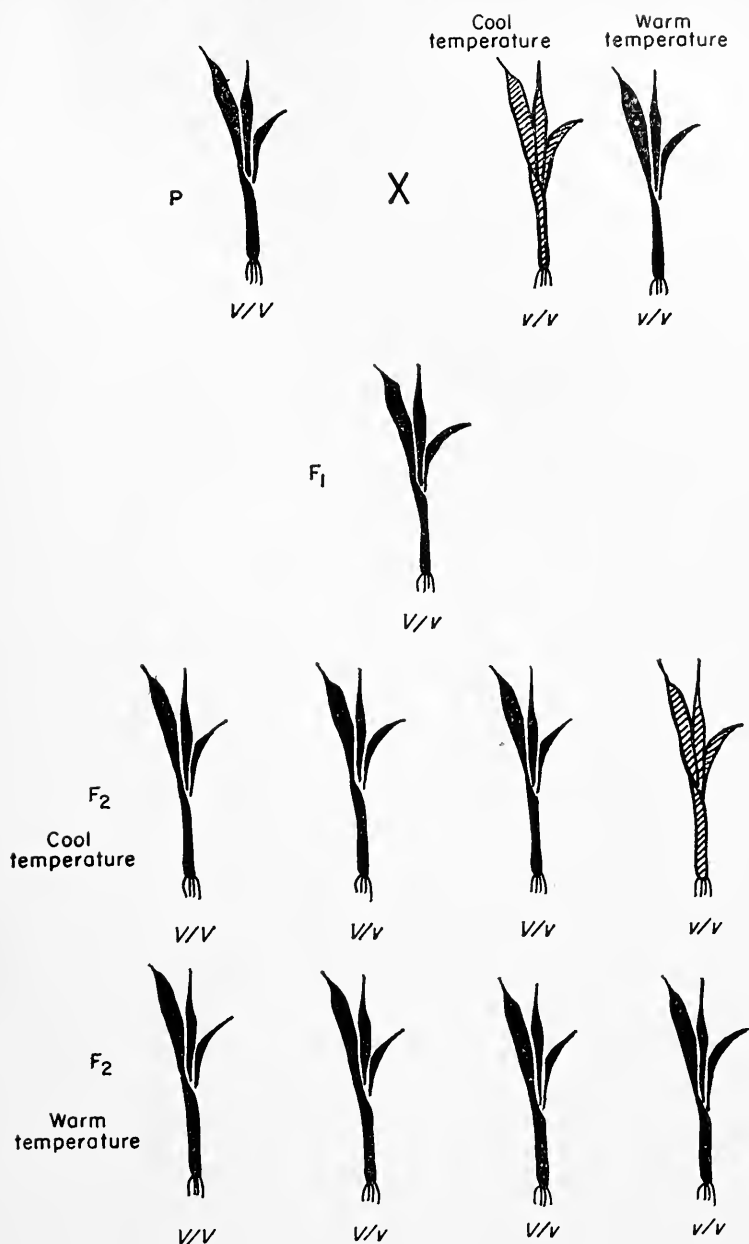


FIG. 6-1 Diagram of cross between normal corn V/V and virescent v/v . In cool temperatures virescents appear greenish yellow, but later turn green. In warm temperatures they appear green and seedlings are indistinguishable from normal $V/-$.

compared to an automobile capable of a speed of 150 miles an hour. On an extremely rough and winding road (the environment) speeds in excess of 25 miles an hour might be virtually impossible.

To change the metaphor, the hybrid corn breeders have developed hybrids capable of yielding 300 bushels an acre. Yields slightly in excess of this have been obtained only twice to date. Such a 300-bushel corn may produce only 100 bushels if supplied with limited fertilizer, or it may give zero bushels if a severe drought should follow germination of the seed. In this instance we might say the environment was more important than the heredity, because a 300-bushel corn and a 100-bushel corn would have exactly the same yield—zero.

DIFFERENCE PRODUCED BY ENVIRONMENT IN IDENTICAL TWINS

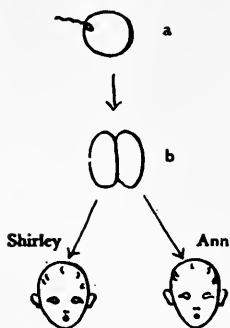
Identical twins have been used in studying the influence of heredity and environment upon the expression of hereditary traits. Identical twins are those derived from a single fertilized egg that divides into two cells. The two individuals that result should have genes and chromosomes essentially alike. Such twins are remarkably similar in phenotype, so that it is often difficult to distinguish one from the other.

A good illustration of the influence of environment on the same genotype is found in Auerbach's *Genetics in the Atomic Age*. Her illustration of identical twins with the same genes for freckling, but with different phenotypic expression, seems quite pertinent. The girl who worked indoors developed comparatively few freckles, while the one who worked out in the sun developed many (Fig. 6-2). In this case the potentialities to produce freckles was acted upon by sunlight in the environment to produce different phenotypic expressions.

ENVIRONMENT AND FLOWER COLOR

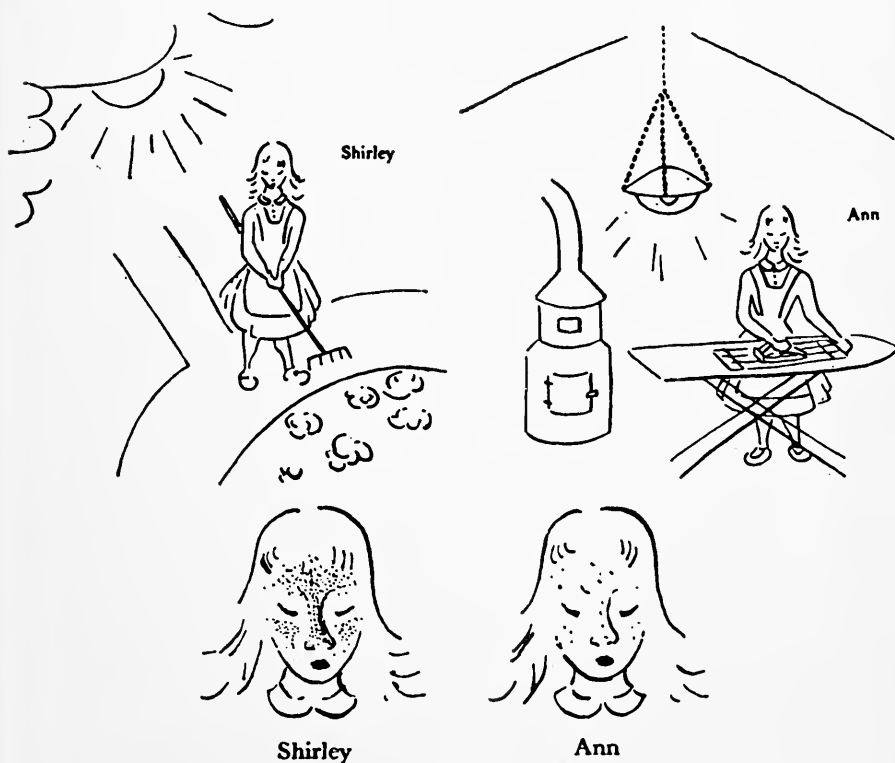
The soil in which certain flowers are grown can be influential in the color that develops. For example, the beautiful blue hydrangeas that everyone admires so much are blue because they are grown in an acid soil. If planted in an alkaline or neutral soil the flowers are an off-white, or faintly pink, not nearly so desirable as the deep blue. Nurserymen are careful to cultivate hydrangeas in an acid soil and gardeners apply aluminum sulphate to the soil to lower the pH so that blue blossoms will develop. In this case the environment is the determining factor in a plant characteristic.

In one particular mutant of the primrose, *Primula sinensis*, the color of the developing flower is dependent on temperature. If the plants are grown at room temperature, the flowers are bright red; at high temperature they are white.



The fertilized egg from which both twins develop.

The egg has divided into two cells with the same genes. These cells become separated, and each develops into one of the twins.



Courtesy of Charlotte Auerbach; and Oliver & Boyd

FIG. 6-2 Influence of environment upon freckling. Identical twins, with same genes for freckling, show difference in amount of marking, because one (Shirley) spent more time in the sun, which accentuates condition.

SUN RED COLOR IN CORN

In maize, Emerson analyzed the genetics of plant colors extensively. He found three genes responsible for the development of a purple anthocyanin color in all parts of the plant. These genes he labeled as follows:

A = for anthocyanin production. The allele a interrupts the development of anthocyanin.

B = an intensifying gene, called by Emerson the "booster" gene.

Pl = in dominant condition produces a purple plant, in presence of A and B .

The pl allele is known as the gene for "sun red." In the presence of sunlight the genotype $A/- B/- pl/pl$ produces a red color. This can be prevented if the plant or any of its parts is covered to exclude the sunlight. If a black cloth is placed over a developing ear, no red develops on the husks, which remain green.

The author demonstrated several years ago that the development of color could be inhibited if either red or orange cellophane were wrapped around the ear of corn. Both of these screen out the rays in the blue-violet end of the spectrum, demonstrating conclusively that it is these rays that are biologically active in the development of red color.

In this case the red was prevented by altering the environment. The prevention of color could also be brought about by altering the heredity. By replacing the B allele with a b , the plant would then have the genotype of $A/- b/b pl/pl$. Its phenotype would be a dilute sun red, which appears as a green plant, and has no potential for producing the sun red color, even under the brightest sunlight.

ENVIRONMENT AND MICROORGANISMS

The biochemical mutants produced and observed in some of the microorganisms provide excellent examples of the interaction of environment and heredity. These mutants are now well known in the bread mold, *Neurospora*. The mutant lacking the ability to synthesize arginine can grow and flourish if arginine is added to the culture medium.

An extreme example of this interdependence is found in the bacterium, *Escherichia coli*. By treating these bacteria with streptomycin, a strain was developed that was resistant to this potent antibiotic. In fact, one mutant was isolated that was not only resistant to the killing action of streptomycin, but actually required it in the culture medium. Here was a marked change in the heredity-environmental relationship by the isolation of the mutant requiring the drug in its diet.

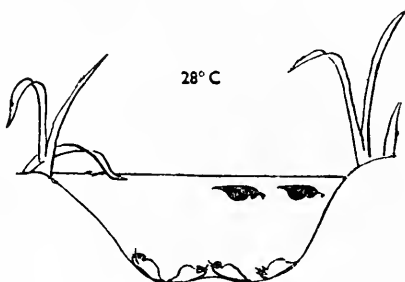
TEMPERATURE-RESISTANT MUTANTS IN DAPHNIA

The little water flea, *Daphnia*, is a common inhabitant of ponds and pools. It is adapted to a temperature of about 20° Centigrade and dies when the temperature rises to about 27°. In the laboratory a mutant arose that requires temperatures from 25° to 30° for survival. This new mutant

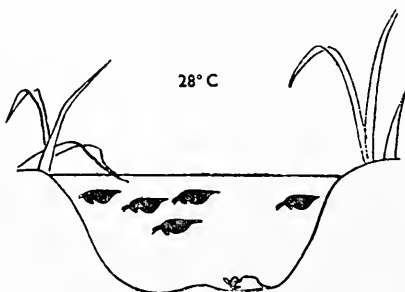
At 20° C. mutants which occasionally arise will die.



When the temperature rises to 28° C. the normal animals die and the mutants survive.



At a constant temperature of 28° C. the population consists only of mutants.



Courtesy of Charlotte Auerbach; and Oliver & Boyd

FIG. 6-3 Temperature sensitivity in water flea, *Daphnia*. These mutants (solid drawing) are unable to survive in water of 20°C and live only if placed in 28°C water. In such water they are the only survivors.

would thrive in warmer temperatures, while the normal strain would all be killed (Fig. 6-3).

TEMPERATURE DEPENDENCE OF COLOR IN CATS AND RABBITS

Siamese cats and Himalayan rabbits are born white and later develop black pigment at the extremities—the feet, nose, ears, and tail. Apparently the color is developed as a result of an enzyme that is inactivated at high

temperatures. The animals *in utero* have the body temperature of the mother and consequently develop no color. As the animals develop, the color is produced *only* at the extremities where the temperature is somewhat lower than the body temperature. An experiment with the Himalayan rabbit has shown that, if the hair was shaved off of a portion of the body, and the animal kept in a cool place as it grew back, the hair was colored. This genotype of rabbit is conditioned by an allele in the *C* series, one designated as c^h . Animals homozygous for c^h/c^h or heterozygous for c^h/c^a have the Himalayan phenotype.

DAY LENGTH AND FLOWERING

The length of day has a profound effect on the time that certain flowers bloom. For example, chrysanthemums bloom in the fall of the year when the days are shorter than the nights. Florists commonly make a practice of covering beds of chrysanthemums so that they will flower earlier (Fig. 6-4).



Courtesy of S. L. Emsweller;
and the U. S. Department of Agriculture

FIG. 6-4 Chrysanthemums may be forced to bloom earlier by covering plants to shorten day. Chrysanthemum variety Mamouth. Plant on left grown on 8-hour photo period. Plant on right, 16-hour photo period.

Several years ago I discovered a mutant in corn that failed to produce ears or tassels during the regular growing season (Fig. 6-5). However, in the fall, when we were harvesting the ears of the normal plants, these mutant types were still green and they grew until killed by frost. When some of the mutant plants were transplanted to the greenhouse, they continued to grow and produced both silks and tassels, but only after the days had become much shorter than the nights. This is caused by a recessive gene which we called *indeterminate* because the plants kept on growing without the terminal inflorescence, the tassel. The gene symbol is *id*. Plants of the *id/id* genotype appear in about one-quarter of a segregating population. Similar indeterminate plants occur in tobacco. They also are conditioned by a recessive gene.

INTERNAL ENVIRONMENT AND GENE ACTION

So far we have discussed the effect of the external environment on the phenotypic expression of hereditary characters. We should also remem-

ber that any gene is affected by the internal environment, the cellular constitution of the organism. Any mutant gene produces its effects usually in a cellular background of normal genes at all the other loci. For example, in *Drosophila* the gene for white eye inhibits the development of color when homozygous in females w/w , or "hemizygous" in males w/Y . But all the other genes are necessary, not so much for the production of a white eye, as for the



Courtesy of Connecticut Agricultural Experiment Station, New Haven

FIG. 6-5 Indeterminate corn. These plants bloom only in short days. Normal (+) plants harvested before id/id plants flowered. A single gene (id/id), when homozygous, causes this response to length of day.

development of a normal *Drosophila* in which the white eye can be expressed.

All genes have a dual function. First, they are necessary for the well being of the organism. If any substantial portion of these genes is lost or missing, the organism fails to develop properly, or it may die if sufficient portions of the chromosomes are absent.

That the w/w genotype can produce a white eye is evidence that we are studying the effect, not of a single gene, but of a "single gene difference" between the white-eyed fly and its normal counterparts. It is as though we had

a long chain of thousands of links, all necessary for the development of the red pigment characteristic of the wild eye in *Drosophila*. The gene for white eye (w) is but one link in the chain. However, breaking the chain at the white "locus" (location or site) of the w mutant interrupts the development of pigment, and a white eye results.

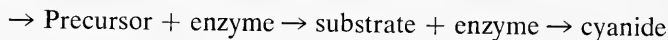
A similar situation exists in the inheritance of corn in which genes for white seedlings have been found. These genes for albino, or white seedlings (w), interfere with normal chlorophyll production, and white seedlings result. Since these seedlings contain no chlorophyll, they are unable to manufacture food by the process of photosynthesis and therefore soon die. Under normal growing conditions the genes are lethal. However, if the environment is altered by feeding sucrose through the leaves, plants can be kept alive, even to maturity.

In corn, more than a dozen of these white mutants have been analyzed and shown to be different genetically. Perhaps the actual number of sites in the corn chromosome capable of mutating to an allele that blocks the production of chlorophyll is many times greater. Hence, to produce a chlorophyll molecule requires the cooperative effort of many genes. Failure of any member of this team to do its part results in a plant with no chlorophyll.

This is another example of the dual function of the gene; its primary function is a team effort in producing a healthy individual. This task can be accomplished by the normal allele or one of several of the mutant types. A healthy, viable *Drosophila* can be produced equally as well with the w allele present in both chromosomes as if the fly had the plus allele present and produced a wild fly.

CYANIDE IN WHITE CLOVER

A specific case of the cooperation of factors in the internal environment is the production of cyanide in white clover. Two complementary genes are responsible for the production of cyanide. One of these genes accounts for a substrate on which an enzyme produced by the other gene can act. If cyanide results from a stepwise process we might visualize it as follows:



If gene A conditions the production of the first enzyme and gene B conditions the production of the second, then the failure at either A or B would result in no cyanide production. Such failures we can label either a or b , meaning that a recessive gene blocks the production of one or the other of these enzymes. Either of these recessive genes in the homozygous condition a/a or b/b alters the internal environment drastically, and manifests itself in a striking manner.

SEXUAL INFLUENCE ON INTERNAL ENVIRONMENT

Some of the most striking manifestations of differences caused by internal environment are to be found in characters whose expression differs in the two sexes. These are sometimes referred to as sex-influenced characters. They may be more appropriately regarded as interactions between the genotype and the cellular environment in which the genes act. The different expressions are probably caused by varying amounts of male and female sex hormones.

PATTERN BALDNESS IN MAN

Although there are several types of baldness in man, the one we are concerned with is the type in which the hair gets progressively thinner on

Table 6-1. Phenotypic Expression of Genotypes for Baldness

GENOTYPE	PHENOTYPES	
	Men	Women
<i>B/B</i>	bald	bald
<i>B/b</i>	bald	non-bald
<i>b/b</i>	non-bald	non-bald

top of the head until little or none is left. This leaves the crown bald and just a fringe of hair below. Apparently this type of baldness is due to a single

Table 6-2. Progeny Resulting from the Marriage of a *B/b* Woman (Non-bald) and a *B/b* Man (Bald)

P		
<i>B/b</i> woman (non-bald) × <i>B/b</i> man (bald)		
F ₁		
♀ gametes ♂ gametes	<i>B</i>	<i>b</i>
	<i>B</i> bald ♂♂ bald ♀♀	<i>B/b</i> bald ♂♂ non-bald ♀♀
	<i>b</i> <i>B/b</i> bald ♂♂ non-bald ♀♀	<i>b/b</i> non-bald ♂♂ non-bald ♀♀

mutant, but the expression of the heterozygote is different in males and females. Table 6-1 lists the different phenotypes in men and women.

Table 6-2 shows the progeny that would result from the marriage of two people heterozygous (B/b) for baldness. This may appear to be a reversal of dominance in males and females. It is perhaps best understood if we realize that the expression of the heterozygous class is altered by the cellular environment (in this instance, the hormonal constitution).

INHERITANCE OF HORNS IN SHEEP

A similar case is that of the inheritance of horns in sheep. Some breeds, the Dorset for example, have horns on both sexes. Others, such as the Suffolk, have no horns on either sex. In a cross of these two breeds, the males have horns, the females none. This is shown in Table 6-3.

Table 6-3. Inheritance of Horns in a Hybrid of Dorset and Suffolk Sheep.

P								
Dorset (H/H) horned \times Suffolk (h/h) hornless								
F_1								
H/h	horned males, hornless females							
F_2								
$\begin{matrix} \nearrow & \sigma^7 \text{ gametes} \\ \searrow & \end{matrix}$	H	h						
$\begin{matrix} \nwarrow & \text{♀ gametes} \\ \nearrow & \end{matrix}$	<table><tr><td>H</td><td>H/H horned $\sigma^7 \sigma^7$ horned $\text{♀ } \text{♀}$</td><td>H/h horned $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$</td></tr><tr><td>h</td><td>H/h horned $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$</td><td>h/h hornless $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$</td></tr></table>	H	H/H horned $\sigma^7 \sigma^7$ horned $\text{♀ } \text{♀}$	H/h horned $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$	h	H/h horned $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$	h/h hornless $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$	
H	H/H horned $\sigma^7 \sigma^7$ horned $\text{♀ } \text{♀}$	H/h horned $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$						
h	H/h horned $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$	h/h hornless $\sigma^7 \sigma^7$ hornless $\text{♀ } \text{♀}$						

Here again it is the heterozygous class that has a different phenotypic expression in males and females. Apparently the hormonal constitution of the individual can alter the phenotypic expression of the heterozygous individuals.

INHERITANCE OF COLOR IN AYRSHIRE CATTLE

The third case of this type of inheritance is that of the color of the spots in Ayrshire cattle. These spots may be either red or mahogany. The

inheritance is shown in Table 6-4. The heterozygous class has a different phenotypic expression in males and females. In all three examples cited, the fact that the heterozygous individuals have a different phenotypic expression in males and females gives an apparent effect of reversal of dominance in the two sexes. This should make us realize that manifestations of dominance are extremely sensitive to environmental conditions, in these cases the internal, cellular, hormonal constituents.

Table 6-4. Inheritance of Red and Mahogany Spots on White Background in Ayrshire Cattle

P		
M/M mahogany ♀ × m/m red ♂		
F ₁		
M/m mahogany ♂ ♂ red ♀ ♀		
F ₂		
♀ gametes \ ♂ gametes	M	m
M	M/M mahogany ♂ ♂ mahogany ♀ ♀	M/m mahogany ♂ ♂ red ♀ ♀
m	M/m mahogany ♂ ♂ red ♀ ♀	m/m red ♂ ♂ red ♀ ♀

That a capital letter has been used to designate one allele and the small letter for the other may be confusing, since the capital letter is usually used to denote a dominant character and the small letter to indicate recessiveness. Sometimes scientific terminology is inadequate to describe what is happening, and the student must realize that all biological processes do not fit into our neat little man-made compartments.

CONCLUDING REMARKS

Both the external environment in which the plants or animals are grown, and the internal or cellular environment in which the genes act may alter drastically the phenotypic expression of the various genotypes. Concerning the internal, or cellular, environment in which the genes operate,

the genes have a dual function. First, they contribute to a healthy individual and, second, produce a characteristic phenotypic expression. In some instances the hormonal environment causes different phenotypic expression of heterozygotes in males and females.

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PROBLEMS

6-1. Define the following terms:

arginine synthesis	pastel maize
cyanide production	pattern baldness (man)
enzyme inactivation at high temperature	pH
external environment	photoperiodism
heredity	precursor
Himalayan rabbit	Siamese cat
hormonal difference in horn production in sheep	streptomycin dependent <i>E. coli</i>
identical twins	substrate
indeterminate corn	sun-red corn
internal environment	temperature-sensitive <i>Daphnia</i>
mahogany spots (Ayrshire)	virescent
monozygotic twins	white eye <i>Drosophila</i>
	white-mutable maize
	white seedlings corn

6-2. Identify the following scientists, giving a major contribution of each, with an approximate date.

Auerbach, Charlotte
Emerson, R. A.

6-3. A bald-headed man, whose father and mother were both bald, married a non-bald woman whose mother was bald, but whose father was not. From this marriage were born eight children, four boys and four girls. Write the genotypes of the grandparents, the parents, and the children, assuming an equal distribution in gamete formation. Give the phenotypes of all eight children upon reaching maturity.

6-4. A purple corn plant ($A B Pl$) was crossed by a dilute sun-red ($A b pl$), normally classified as green. Give the full genotype of the parents and the F_1 , showing the phenotype of the F_1 . Show by a zygotic checkerboard, or by the branching method, the phenotypes expected in the F_2 with the proportions of each.

- 6-5. The developing ear is a good place to observe plant colors in corn. How would your ratio be modified if all the developing ears were covered by red cellophane when quite young? What proportion of purple, sun-red, and green husks would you get?
- 6-6. In determining acidity a pH of 7.0 is neutral, higher values are basic, and lower values acid. What color of flowers would you expect on hydrangea plants grown in soil with a pH of 5.0? In one with a pH of 8.5?
- 6-7. If the hair were shaved off a small portion of the skin of a Himalayan rabbit and an ice pack kept on this spot while the hair grew in, what would be the color of the spot?
- 6-8. A farmer who bred Ayrshire cattle wished to design a breeding scheme so that bull and heifer calves could be distinguished by a difference in color. As a geneticist, how would you advise him to do it?
- 6-9. The same farmer raised sheep. He had a preference for horned males and hornless females. Advise him how these results could be obtained.

Sex-Linked Inheritance in *Drosophila*, Cats, Poultry, and Man

IN PREVIOUS CHAPTERS the inheritance of a number of characters conditioned by a single gene has been discussed. Some of the characters studied in *Drosophila* include such eye colors as brown and scarlet, the body colors ebony and black in contrast to normal gray, and the wing character vestigial. In all of these characters it makes no difference whether the mutant being studied is introduced into the hybrid by way of the male or female. Reciprocal crosses are alike. The mutants, being recessive, do not appear in the F_1 but reappear in one fourth of the F_2 population in both males and females.

This is not true for the white eye condition, one of the first mutants discovered by Morgan in his laboratory at Columbia University in the early years of the present century. This mutant appeared in a single male *Drosophila*; the phenotypic expression was completely white in contrast to the dark red eye of the wild-type stock. Morgan mated the white-eyed male to a wild type, red-eyed female. The F_1 offspring all had red eyes. These were interbred and produced in the F_2 all red-eyed females and both red-eyed and white-eyed males. When white-eyed males were mated to their sisters, some produced equal numbers of white-eyed males and females. When some of these white-eyed males and females were mated, a pure breeding stock of white eye was obtained. In this example a new type of inheritance is seen. The white eye appears in different frequency among the males and females of the segregating populations. This is all readily explained when it is understood that the gene for white eye w and its normal allele w^+ or $+$ in the wild fly are located in the X chromosome, one of the sex-determining chromosomes.

It is now well known that, in *Drosophila* and in many mammals, one pair of the chromosomes is concerned with the determination of sex. These chro-

mosomes can be observed readily under the microscope. In the female *Drosophila* there are four pairs of chromosomes. Each pair is distinguishable morphologically from the other, but homologous members of a pair are essentially alike. In a male, however, one pair of chromosomes shows considerable difference between the two. (Fig. 7-1.)

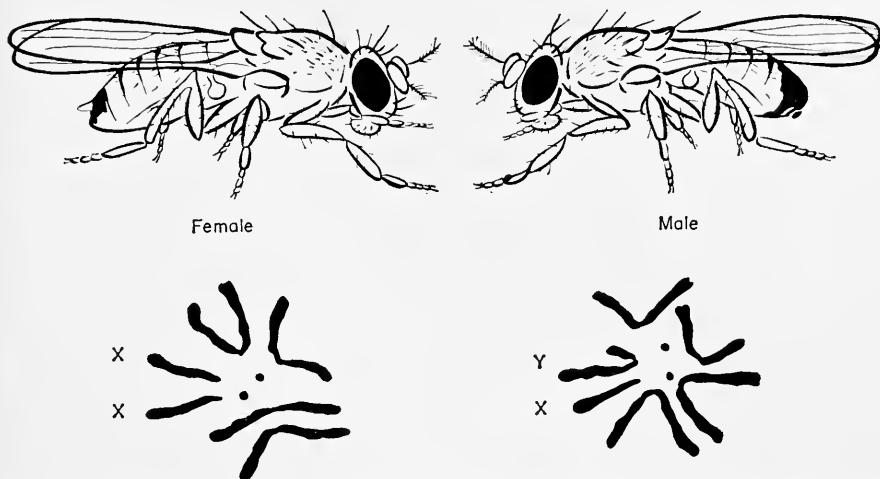


FIG. 7-1 Female and male *Drosophila melanogaster*, showing diagrams of metaphase chromosomes below. Note the difference in X and Y chromosomes, also sex comb (black dot) on foreleg of male but not female.

This heteromorphic pair of chromosomes is concerned with the determination of sex. In *Drosophila* the male is the *heterogametic* sex (sex chromosomes unlike), while the female is the *homogametic* sex (sex chromosomes alike and hence capable of producing only one kind of gamete).

The sex or X chromosome in *Drosophila* contains a rather large number of genes, all of which show the type of inheritance found by Morgan for white eyes. Apparently the Y chromosome is composed mostly of *heterochromatin*. It neither contains recessive mutants, nor dominant alleles of any of the genes in the chromosome. Hence an X/Y fly, a male, has its phenotype determined by the genes in its X chromosome which consists mainly of *euchromatin*, the gene-bearing portion. Since it has only one X chromosome, it is often referred to as *hemizygous*.

ONE WHITE-EYED MALE MUTANT IN DROSOPHILA

Now let us return to the white-eyed male mutant studied by Morgan. The first white mutant observed was a male. This is understandable since the male has only one X chromosome and there is no gene in the Y chromosome to mask the expression of white. The white mutant observed

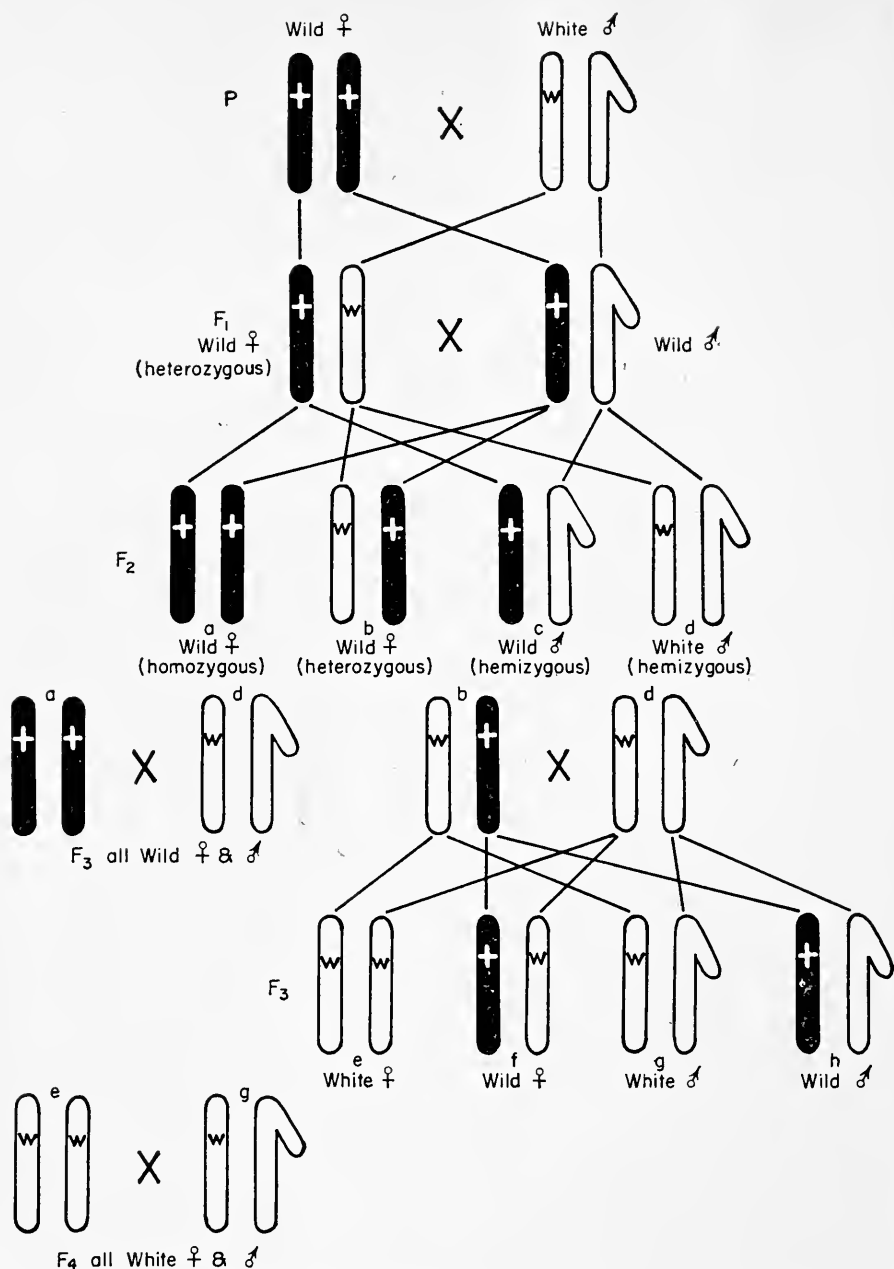
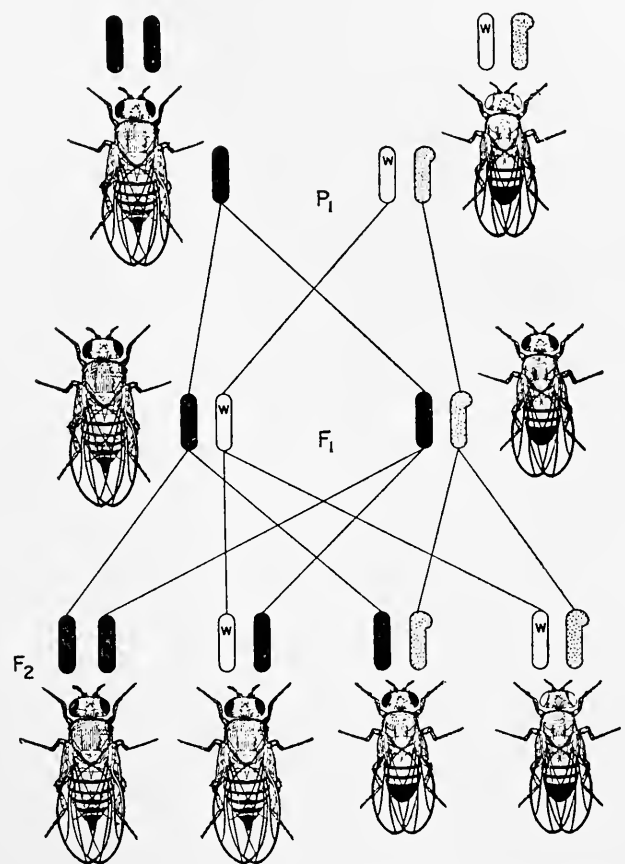


FIG. 7-2 Diagrams showing matings made by Morgan to establish inheritance of gene for white eye (*w*). Original white-eyed ♂ mated to wild ♀ produced F₁ with ♀'s and ♂'s wild type. Mating of two F₁'s produced F₂ with two types of ♀'s *a* and *b*, and two types of ♂'s *c* and *d*. The *d* fly (♂) had white eyes. Mating of *a* × *d* gave all wild F₃. Mating of *b* × *d* gave F₃ shown in diagram. Mating of F₃ *e* × *g* gave all white progeny, establishing a homozygous strain of the white-eyed mutant.

got his X chromosome from his mother. Sex-linked genes are regularly transmitted from mother to son, and never from father to son. The mutation from wild (w^+) to white (w) occurred by chance, but it was not chance that led Morgan to a careful analysis of the inheritance of this character. It was a



From T. H. Morgan, *The Theory of the Gene*, by courtesy of Yale University Press

FIG. 7-3 Diagram of mating of red-eyed female by white-eyed male *Drosophila*. The X chromosome carrying the gene for red eye is represented by the black rod; the X chromosome carrying the gene for white eye, by the open rod; and the white recessive gene carried in the chromosome, by small *w*. The Y chromosome is stippled.

carefully planned experiment. When the mutation occurred, Morgan was ready to take advantage of it. As Pasteur has said, "Chance favors the prepared mind." The one white-eyed male mutant, in the hands of a capable investigator, led to an explanation of all sex-linked characters in *Drosophila*. These findings are also applicable to other organisms, as will be discussed presently.

The matings made can be illustrated by a diagram showing the X chromosome different morphologically from the Y chromosome. The diagram of the matings made by Morgan is found in Fig. 7-2.

This same type of inheritance, in which a white male fly is mated to a homozygous wild-type female, is shown in Fig. 7-3. This is one of Morgan's illustrations in *The Theory of the Gene*.

In the reciprocal cross, when a white female is mated to a wild-type male, different results are obtained (Fig. 7-4). In this F_1 , all the males are white.

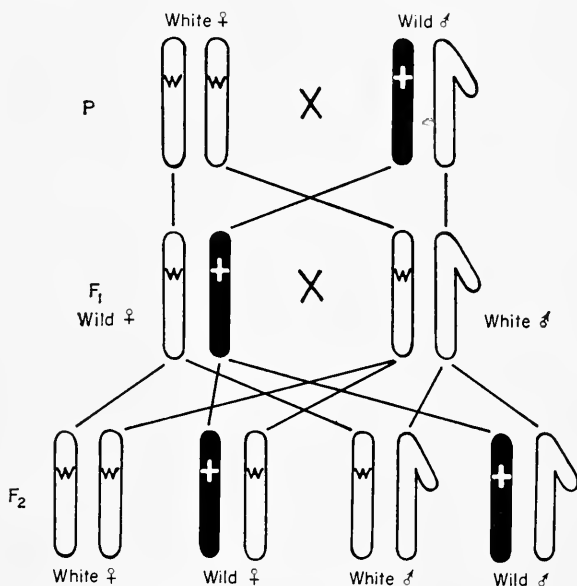


FIG. 7-4 Sex-linked inheritance in *Drosophila*.

Each received his X chromosome from the mother and could have only one kind of X chromosome, which contained the gene for white (*w*). The females are all of the wild type, since they received one of the X chromosomes from the father. In such a mating the X chromosome of the F_1 male carries *all* the recessive genes present in the female parent. Hence an F_2 of such a mating actually represents a testcross. This is quite useful in studying linkages, as will be discussed in Chapter 8. It will be noticed that in both males and females, white-eyed and red-eyed flies appear in equal numbers, a typical test-cross ratio.

TORTOISE-SHELL CATS

In Chapter 1 we discussed the sex-linked nature of the inheritance of tortoise-shell cats, those with blotches of black, yellow, and white in their coats. It was also stated that such cats were always females. This phenotype

is produced in the heterozygous condition of the gene determining black or yellow coat color. If we designate black color as B/B , or preferably $+/+$, and the yellow color as b/b , then the F_1 females would be of the constitution $+/b$, and would be neither black nor yellow, but tortoise-shell (Fig. 7-5). Note

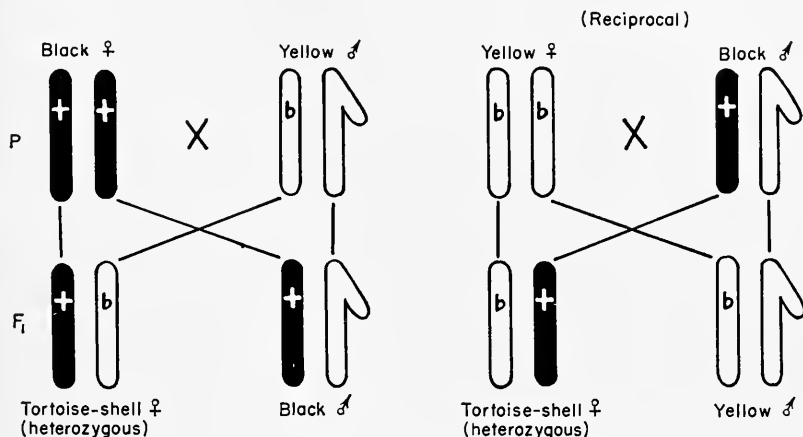


FIG. 7-5 Sex-linked inheritance of coat color in cats.

that the females are the same regardless of the way the cross is made. This is because *neither* black nor yellow is dominant, but the heterozygote is distinguishable from both homozygotes. The male, being hemizygous, can be either yellow or black, but not both. It should be noted that occasional tortoise-shell male cats have been observed. They have been sterile, however, most likely because of an additional X chromosome. These cases will be discussed in Chapter 13 on sex determination.

HEMOPHILIA AND COLOR-BLINDNESS IN MAN

In man there are 46 somatic chromosomes, 23 pairs in the reduction division. In somatic tissue it is readily observable that these 46 chromosomes really represent two sets of 23 chromosomes each. The pairs of homologous chromosomes are essentially alike, all except one pair in which one chromosome is much smaller than its homologue. This is the Y of the XY pair (Fig. 7-6).

A woman has two X chromosomes which are alike, while a man has one X and the smaller Y. Apparently this smaller Y chromosome contains few genes. All genes located in the X chromosome show the same type of inheritance as the sex-linked characters in *Drosophila*. One of these is the gene for *hemophilia*, located in the X chromosome. It is recessive, and its expression is completely masked by the dominant allele. Hence, a woman may be carrying a gene for hemophilia (h) and be capable of transmitting it to half of her sons,



Courtesy of E. H. Chu

FIG. 7-6 Somatic chromosomes in man, with homologous chromosomes arranged in pairs. Sex is determined by the XY pair, in this case a ♂. A ♀ would have two X chromosomes.

without showing any visible effects of the condition. Individuals affected with hemophilia lack an agent that causes the normal clotting of blood after even a minor cut. Prolonged bleeding may ensue, and the condition is usually fatal at an early age.

By analogy with the sex-linked characters in *Drosophila*, males are much more often affected since they have only one X chromosome. All genes in this chromosome are in the hemizygous condition. In a female, however, a recessive gene in one X chromosome may be masked by a dominant allele in the other X chromosome.

Since hemophilia is usually fatal at an early age, it is evident that most cases

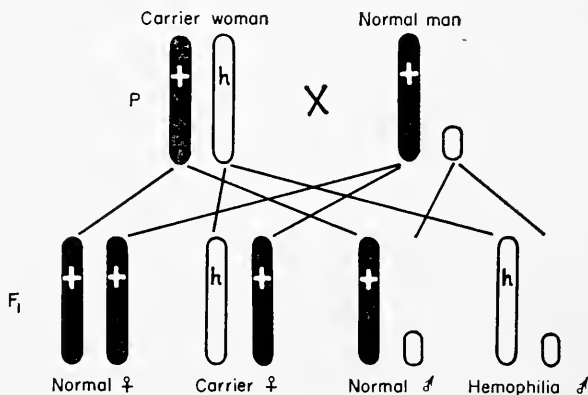


FIG. 7-7 Sex-linked inheritance of hemophilia in man. This condition is transmitted from heterozygous mother $+/h$ to one half of sons.

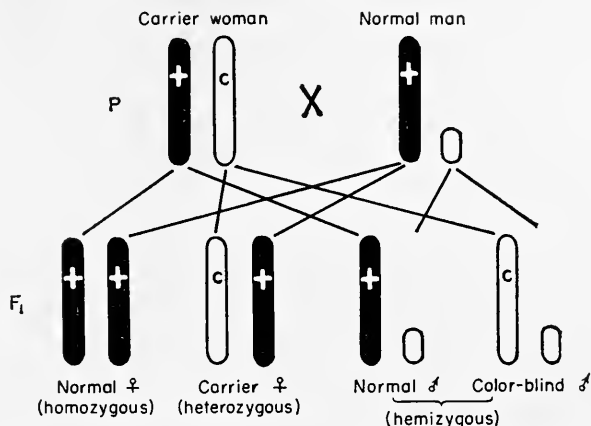


FIG. 7-8 Sex-linked inheritance of red-green color blindness in man. Heterozygous mother produces color blindness in one half of sons, but not in daughters.

of it are transmitted from mothers who are carriers, i. e., heterozygous H/h . Such a mother would transmit this gene to one half her sons regardless of the constitution of the father (Fig. 7-7).

The gene for red-green color blindness is also in the X chromosome in man, and hence it follows the same mode of inheritance as does the gene for hemophilia. There is a difference, however, in that males who are color-blind are fully as viable as those with the dominant allele. Hence they can transmit this gene to their daughters. If such a gene meets with one from the mother, then the daughter will be color-blind. A father *never* can transmit this condition to his son. The inheritance of color blindness is shown in Figs. 7-8 and 7-9.

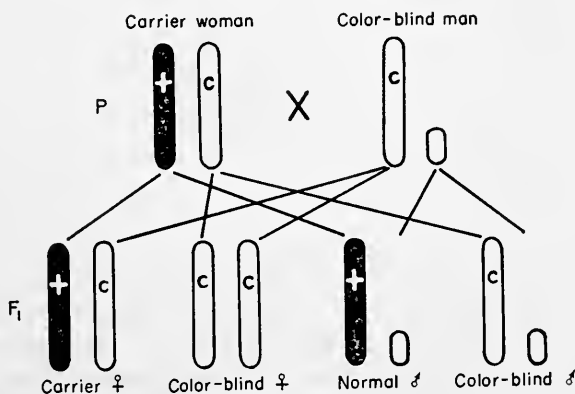


FIG. 7-9 Similar to Fig. 7-8, except carrier woman $+/c$ is mated to color-blind man c/Y . One half of sons *and* daughters are color-blind.

SEX-LINKED INHERITANCE IN POULTRY

In poultry several genes are located in the X chromosome, and their inheritance follows the same general scheme as outlined for *Drosophila*, cats, and man. However, the female is the heterogametic sex. The hen has but one X chromosome and no Y. This is known as an XO condition. She produces

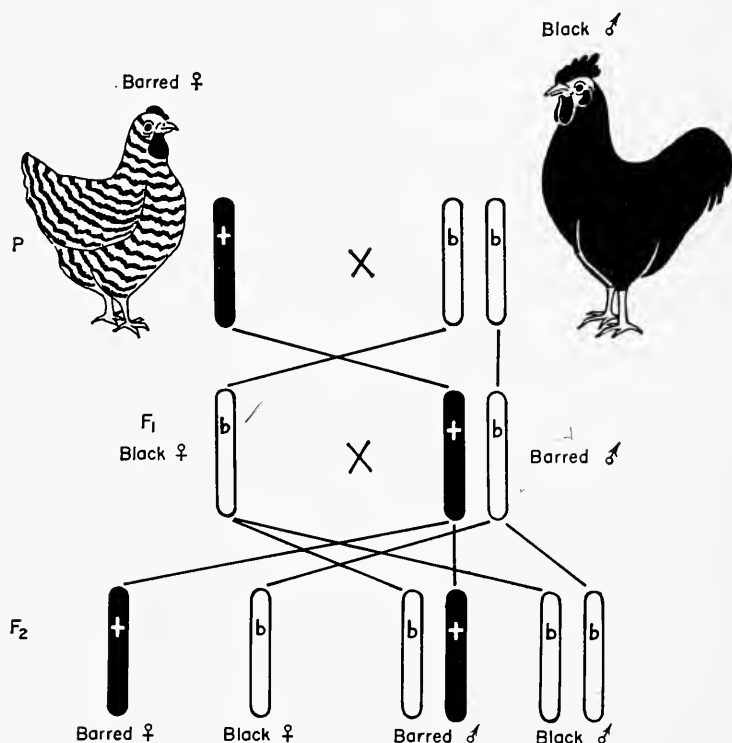


FIG. 7-10 Sex-linked inheritance in poultry. A mating of a barred female to a black male is illustrated. In poultry the female has but one X chromosome with no Y (an X-O condition). The male has two X chromosomes.

two kinds of gametes, one with an X chromosome and one without. The male is the homogametic sex XX, producing only one kind of gamete, that with the X chromosome. This can be illustrated best by the gene for barred feathers, found particularly in the Barred Plymouth Rock breed. Instead of solid coloring, the feathers are banded with "bars" of black on a white background. This gives the characteristic barred effect. This inheritance is shown in Figs. 7-10 and 7-11.

CONCLUDING REMARKS

Sex-linked characteristics of animals discussed in this chapter show a mode of inheritance different from that presented previously. The genes for characters showing sex linkage are located in the X chromosome of the XY pair concerned with the determination of sex. In *Drosophila* and most mammals, including man, the female is the homogametic sex (XX), while the

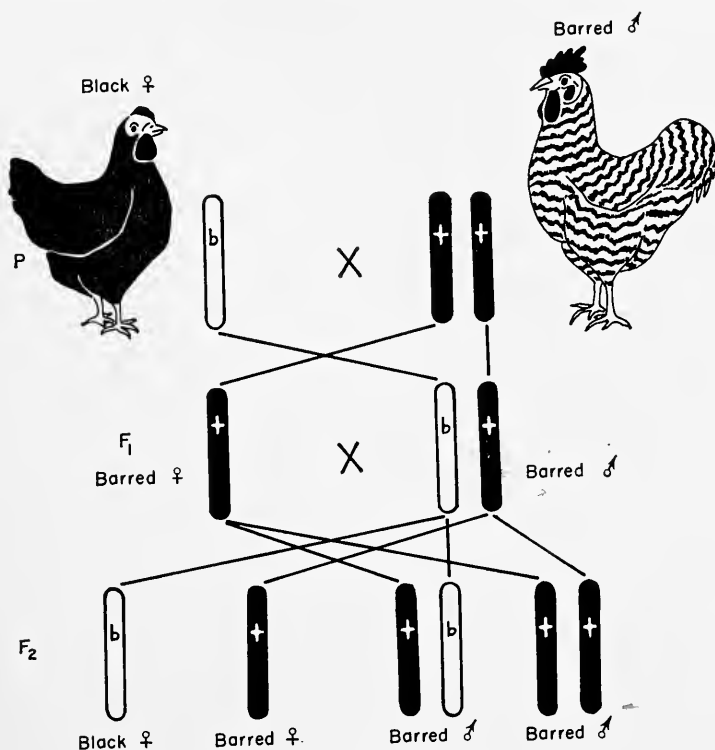


FIG. 7-11 Sex-linked inheritance in poultry. Similar to Fig. 7-10, except that a black female is mated to a barred male.

male is heterogametic (XY). The Y chromosome is composed mainly of heterochromatin with few genes. It contains nothing to mask the expression of any character whose determiner (gene) is in the X chromosome. Therefore, one X chromosome of XY individuals alone determines the inheritance of genes located in it. XY individuals are hemizygous, and transmit only the X chromosome to their daughters. In *Drosophila* and mammals, males transmit an X chromosome to their daughters, never to their sons. In poultry, females transmit an X chromosome to their sons, never to a daughter.

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PROBLEMS

- 7-1. Define the following terms:
- | | |
|---------------------------|-------------------------------|
| Barred Plymouth Rock | sex linkage |
| hemophilia | testcross |
| heterogametic sex | tortoise-shell cat |
| heteromorphic chromosomes | white eyes— <i>Drosophila</i> |
| homogametic sex | X chromosome |
| linkage | XO condition |
| red-green color blindness | Y chromosome |
- 7-2. Identify the following scientist, giving a major scientific contribution, with an approximate date.
- Morgan, T. H.
- 7-3. In *Drosophila* the gene for yellow body *y* is in chromosome 1 and shows sex-linked inheritance. Make a diagram showing the parents, F_1 and F_2 , when a yellow female is crossed by a wild male.
- 7-4. Diagram the reciprocal mating. What is the striking difference between the results and those in Problem 7-3?
- 7-5. In poultry it is desirable to be able to distinguish male and female baby chicks. Barred and black baby chicks are different phenotypically. Design a breeding experiment that would enable the poultryman to separate the two sexes.
- 7-6. A red-green, color-blind man marries a normal woman whose father was color blind. They have eight children, four boys and four girls. Assuming an equal distribution of color blindness and normal vision among the children, how many of each would be expected for the two sexes? The recessive gene for red-green color blindness is in the portion of the X chromosome which is not homologous with Y.
- 7-7. A woman whose father was a hemophiliac marries a normal man. If they have eight children, four boys and four girls, how many hemophiliacs would you expect among them? Show the expected results by a diagram.
- 7-8. Another woman with no record of hemophilia in her ancestors marries a man whose father was a hemophiliac. How many bleeders would be expected in a family of four children, two boys and two girls?
- 7-9. A tortoise-shell cat has a litter of eight kittens—two tortoise-shell females, two yellow females, two black males, and two yellow males. What color of tomcat was the sire of this litter?
- 7-10. A black female cat has a litter of five kittens, three tortoise-shell and two black. What is the sex of the kittens? What was the color of the sire of the litter?

Linkage, Multiple Gene Segregation, and Non-Random Assortment

MENDEL'S SECOND LAW of inheritance stated that when two or more "elements" are heterozygous in a hybrid, the segregation of any one is completely independent of the rest. In other words, all the genes are distributed to the gametes at random.

Fortunately for the characters studied by Mendel in the material he was using, the common garden pea, this was true. He was studying the inheritance of seven characters, the inheritance of each being due to a single gene. Genes for all seven characters showed random assortment. It was later demonstrated cytologically that peas have seven pairs of chromosomes. It has been shown genetically that the genes for the characters studied by Mendel are each located in a different member of the seven pairs of chromosomes.

We might say that Mendel was fortunate that the seven characters he chose to work with each had its determiner or gene located in a different chromosome. This is indeed a "long shot" when the probabilities are considered. Let us use different colored marbles to represent the seven chromosomes of the pea. Since there are seven colors in the spectrum, these colors may be used. If we take 100 red marbles to represent chromosome 1, 100 orange for chromosome 2, etc., we will have 100 marbles each of red, orange, yellow, green, blue, indigo, and violet. We have used the number 100 as a minimum of genes possible in any one chromosome. This is a conservative figure, as there are perhaps many more possible sites of mutation in each of the chromosomes.

Let us now mix these marbles of seven different colors in a bag and then, without looking, draw out seven marbles in succession. What are the chances that all seven will be of a different color? It is obvious that our chance of selecting a red marble, or one of any other color, on the first draw is one

seventh, since there are seven different colors. The chance of getting a marble of any other color at the second draw is likewise one seventh, so that the chances of getting a red marble and a green one in two trials is $\frac{1}{7} \times \frac{1}{7} = \frac{1}{49}$. The chance for all seven being different is $(\frac{1}{7})^7 = 1/823,543$, or about one in a million. Surely it was a long shot. Mendel was lucky! And how fortunate we are that he was so lucky.

If Mendel had found an association of two characters he might not have understood it, any more than William Bateson did early in the present century. Bateson was a pioneer in genetic research in England. He discovered "gametic coupling" between two dominant genes in the sweet pea when blue color and long pollen grains showed a gametic ratio $7 BL:1 Bl:1 bL:7bl$. Unfortunately, Bateson had crossed two white varieties that gave a purple, and hence could not tell the parental types producing the odd gametic ratio. He did not associate it in any way with the chromosomes, although this was after Sutton had postulated the plausible theory that the chromosomes were the carriers of the hereditary determiners, the genes. Sutton even wrote that many genes could be within a single chromosome.

Bateson was unable to associate the gametic coupling with physical location of the determiners within a chromosome. If so, how much less likely was it that Mendel could have explained linkage (had he found it) before our knowledge of the chromosomes, and before the laws of inheritance had been established? So we are indeed fortunate the characters studied by Mendel were determined by genes in different chromosomes.

Had Mendel studied the inheritance of one more character, he might have observed an exception to his second law—that of random assortment.

The first significant deviation from random assortment of genes at meiosis was reported in the previously mentioned experiments of Bateson (1905). In a cross between one variety of sweet pea, Emily Henderson, with white flower and long pollen grains and another type with white flower and round pollen grains, he obtained an F_1 with purple flowers and long pollen. The long pollen was dominant and there were two complementary genes for flower color, called *C* and *R*. The F_2 generation produced a ratio of 27 purple : 9 reds : 28 whites, or 36 colored : 28 whites, which is a 9:7 ratio expected if two complementary genes for color are segregating. This type of inheritance will be discussed in Chapter 9.

The important thing for us here is the association between the purple or bluish-colored flowers and long pollen. The experiment showed that these did not segregate at random to give the four gametic classes found in Table 8-1.

Table 8-1. Gametes Produced for Two Genes Segregating in Sweet Peas

BL = blue flower, long pollen
Bl = blue flower, round pollen
bL = red flower, long pollen
bl = red flower, round pollen

The expected ratio for random distribution was 1:1:1:1 for the four classes. The results obtained were the following:

$$7 BL:1 Bl:1 bL:7 bl$$

This phenomenon Bateson called "gametic coupling." We still use the term coupling to denote a linkage between two dominant (or two recessive) alleles. Bateson was at a loss to explain the 7 to 1 relationship. Also he was puzzled in another case in which the two dominants seemed to repel each other. This he called *repulsion*. We still use the term today to denote linkage of a dominant and a recessive, even though there is no repulsion between unlike alleles, but an association of members of different pairs of alleles introduced into the cross in the same chromosome. Some geneticists call a heterozygote in the repulsion phase a *trans-heterozygote* and a hybrid in the coupling phase a *cis-heterozygote*. These names seem more appropriate and in time may replace the less accurate terms.

MORGAN AND LINKAGE

The first logical explanation of non-random distribution of characters in the segregating generation was Morgan's short but classic paper "Random Segregation Versus Gametic Coupling in Mendelian Inheritance," published in *Science* (1911).

By this time there was considerable evidence that certain characters in a number of organisms did not show random assortment in the distribution of their determiners (genes) at germ cell formation. Morgan proposed a simple explanation to account for the known facts. He assumed that genes showing a non-random segregation were located within a single chromosome and that these genes were arranged in a linear series. If two were close together in this linear series, they would show a closer association (linkage) than two genes farther apart. In Morgan's words:

We find coupling in certain characters, and little or no evidence at all of coupling in other characters, the difference depending on the linear distance apart of the chromosomal materials that represent the factors [genes—W.R.S.]. Such an explanation will account for all of the many phenomena that I have observed and will explain equally, I think, the other cases so far described. The results are a simple mechanical result of the location of the materials in the chromosomes and of the method of union of homologous chromosomes, and the proportions that result are not so much the expression of a numerical system as the relative location of the factors in the chromosomes. Instead of random segregation in Mendel's sense we find "association of factors" that are located near together in the chromosomes. Cytology furnishes the mechanism that experimental evidence demands.

Morgan used the term "factor" for the hereditary determiner, a term still used by some geneticists, although "gene" is a more precise term and is used throughout this text. It should be noted that Morgan spoke of the genes *in* the

chromosome rather than *on*, as is sometimes used in genetic writings. The genes are actually in the chromosome, being an integral part of it.

This, in brief, is the explanation of linkage, just as correct today as when proposed by Morgan in 1911. For this and other brilliant researches in *Drosophila*, Morgan was awarded the Nobel Prize in Medicine in 1933, the first geneticist to be so honored.

CYTOLOGICAL BASIS OF LINKAGE

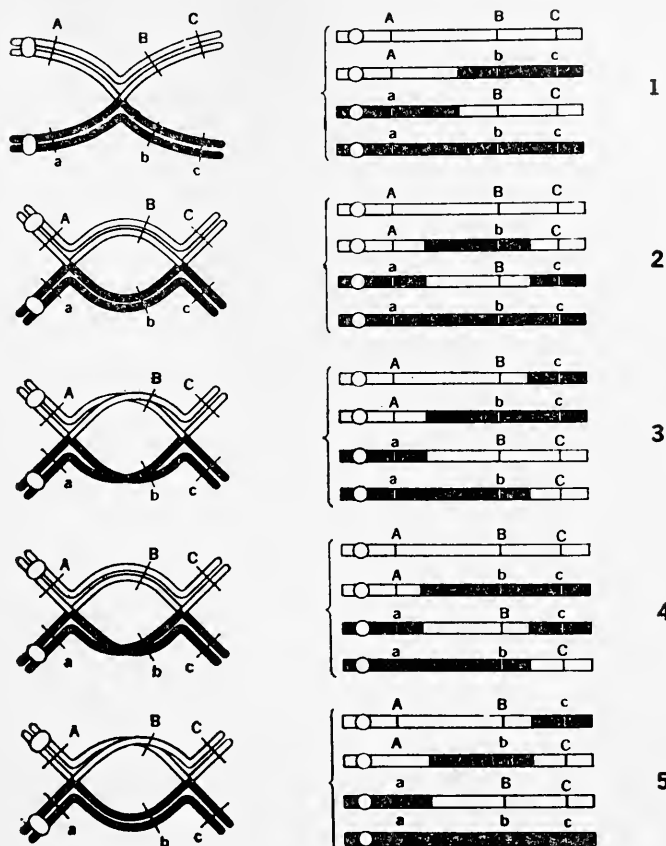
During prophase I of meiosis, the homologous chromosomes pair. Apparently there is an attraction of homologous parts of the two different members of a chromosome pair, and the pairing is extremely precise. Sometime between pachytene (see diagrams in Chapter 2) and diplotene, (two stages in the prophase of meiosis), the two different homologous chromosomes divide with two chromatids each, or four chromatids for each chromosome pair. At about this time the chromatids establish one or more points of contact or exchange per bivalent. This cytological exchange point is known as a *chiasma* (pl. *chiasmata*).

Exchange of equal portions of chromatids occurs at these chiasmata, so that an individual chromatid is not passed on intact, but the new chromatid may consist of parts of two homologous chromatids that have been joined in a chiasma. This is an unusually precise process so that the exchange is between equivalent parts of the original chromatids. There is ample genetic and cytological evidence that the crossing over or exchange is between chromatids and not between whole undivided chromosomes. As a result of this exchange of equivalent segments of chromatids during meiosis, new chromosomes are formed with assortments of genes different from the original chromosomes entering prophase.

Fig. 8-1 shows diagrammatically what takes place when chromatids undergo various types of exchanges at meiosis. The first pair of chromosomes (1) shows one chiasma, the other four pairs show two chiasmata each. In No. 1, there is a single crossover between two of the strands, with the other two strands remaining unchanged. In No. 2, two of the strands show a double crossover and two parental strands. In No. 3, all four of the strands show a single crossover. In No. 4 and No. 5, the end result is one parental strand, plus three showing a single crossover each.

LINKAGE IN CORN

An ear of corn is an excellent subject for observation of linkage. If we have an ear segregating for colored versus colorless kernels, also segregating for well-filled versus shrunken kernels, we would expect in a testcross to obtain equal numbers of the following classes:



Courtesy of Sinnott, Dunn, and Dobzhansky;
and McGraw-Hill Book Company

FIG. 8-1 Chiasma formation in meiosis I (diagrammatic) for a single pair of chromosomes with four chromatids. (1) Single chiasma with single crossover between two chromatids. (2) Two chiasmata with double crossover between two chromatids. (3) Two chiasmata with single crossovers in all four chromatids. (4) Two chiasmata, one parental type chromatid, two with single crossovers, and one with double crossovers. (5) Two chiasmata, one parental chromatid, two with single crossovers and one with a double crossover. Similar to No. 4.

1 colored plump : 1 colored shrunken.
1 colorless plump : 1 colorless shrunken.

Bear in mind that this is a testcross ratio where the products of all gametes are directly observable. The double recessive gamete contributed by the recessive type can in no way mask the expression of any of the gametes contributed by the F_1 plant.

If we assign the gene symbols, A for kernels containing anthocyanin, a for

colorless for the two alleles of the *A* locus, *Sh* for plump, and *sh* for shrunken, the genotypes of the parents can be written as follows:

$$\begin{array}{c}
 \text{P} \\
 \hline
 \text{colored plump } \frac{A\ Sh}{A\ Sh} \times \text{colorless shrunken } \frac{a\ sh}{a\ sh} \\
 \\
 \text{F}_1 \\
 \hline
 \frac{A\ Sh}{a\ sh} \text{ colored plump}
 \end{array}$$

The F_1 might just as well be written $A\ Sh/a\ sh$, which is the typewriter's way of designating $A\ Sh$ over $a\ sh$. The parents would be $A\ Sh/A\ Sh$ and $a\ sh/a\ sh$. This is the system generally used by geneticists for showing that one gene is in one chromosome, and the other allele of this gene is in the homologous chromosome, as A/a . It is just as logical to write $A\ Sh/a\ sh$ as it is A/a , and this system will be used throughout this text.

The testcross of the F_1 hybrid using this designation is $A\ Sh/a\ sh \times a\ sh/a\ sh$.

Four types of gametes produced by the F_1 plant with the four genotypes produced by the testcross are shown in Table 8-2. With random assortment

Table 8-2. Gametes and Genotypes Produced in a Testcross Progeny of $A\ Sh/a\ sh \times a\ sh/a\ sh$

	Gamete	Genotype	Phenotype
Parental	$A\ Sh$	$A\ Sh/a\ sh$	colored plump
	$a\ sh$	$a\ sh/a\ sh$	colorless shrunken
Crossover types	$A\ sh$	$A\ sh/a\ sh$	colored shrunken
	$a\ Sh$	$a\ Sh/a\ sh$	colorless plump

these different phenotypes (and genotypes) would be expected in equal numbers. However, experiments have shown results like those in Table 8-3, assuming a population of 10,000 seeds.

Table 8-3. Experimental Results of Testcross $A\ Sh/a\ sh \times a\ sh/a\ sh$

Genotype	Phenotype
$A\ Sh/a\ sh$ 4990	colored plump
$a\ sh/a\ sh$ 4990	colorless shrunken
$a\ Sh/a\ sh$ 10	colorless plump
$A\ sh/a\ sh$ 10	colored shrunken
Total	10,000

This is a great discrepancy from the expected ratio of 2500 for each class with random assortment, but it is readily understood by Morgan's explanation of linkage. The genes *A* and *Sh* are very close together in chromosome 3 in maize. Consequently most of the gametes are either of the type *A Sh* or *a sh*, the other parental type. About .2% of the gametes are the crossover types

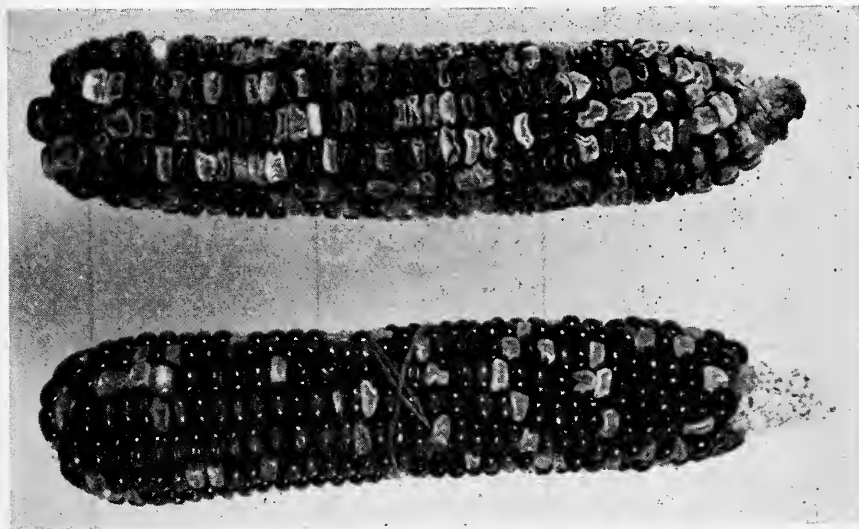


FIG. 8-2 Testcross ear of corn showing linkage of *A Sh₂* and *a sh₂*. (Top) Note that crossovers *A sh₂* and *a Sh₂* are exceedingly rare, only 2. Selfed ear at bottom (one crossover)

a Sh and *A sh*. An ear showing such a segregation may be seen in Fig. 8-2; also in Frontispiece at 12 o'clock. Actually only one crossover type, *a Sh*, was obtained on this ear. Since but one kernel per 500 was expected, it is not surprising that one of the crossover types was absent.

LINKAGE IN DROSOPHILA

Two characters in *Drosophila* showing linkage are white eye and yellow body. The genes for these are both in the X chromosome, discussed in Chapter 7.

The X chromosome is a convenient one for studying linkage. If the cross is made with the double recessive female, the whole F_2 progeny will represent a testcross, because the F_1 male will be a recessive hemizygote. For example, if a yellow-bodied, white-eyed female *yw/yw* is crossed by a normal male $+/+$ $+/Y$, the F_1 male receives his X chromosome *yw* from his mother and the Y chromosome from his father. The genotype will be *yw/Y*, which will be yellow-bodied and white-eyed. The F_1 female is *yw/+* and produces four

classes of gametes, the parental types yw and $++$; also the crossover types $y+$ and $+w$. The gametes are shown in Table 8-4, with the proportion of flies resulting from such an F_2 (which actually is a testcross). Results are given for a population of 1000 males and 1000 females (Table 8-4).

Table 8-4. F_2 Population (Actually a Testcross) Showing Linkage of y and w in X Chromosome of *Drosophila*

♂ gametes		♀ gametes	
		yw	Y
Parental type	yw 49.2%	♀ yw/yw yellow body white eye 492	♂ yw/Y yellow body white eye 492
Parental type	$++$ 49.2%	♀ $++/yw$ gray body red eye 492	♂ $++/Y$ gray body red eye 492
Crossover type	$y+$.8%	♀ $y+/yw$ yellow body red eye 8	♂ $y+/Y$ yellow body red eye 8
Crossover type	$+w$.8%	♀ $+w/yw$ gray body white eye 8	♂ $+w/Y$ gray body white eye 8

These actual numbers would not be expected in a population of exactly 1000 males and 1000 females, but the results of all experiments conducted show that the crossing-over between y and w is 1.5% or 15 per 1000. We used 16 per 1000 in order to avoid the unpleasant thought of splitting a *Drosophila*!

The crossover values for other characters located in chromosome 1, the X

chromosome, have been determined. Some of these values are not nearly as closely linked as white eye and yellow body. If we should make a cross between a yellow-bodied female with normal red eyes ($y+/y+$) and a male with a gray body and vermilion eyes ($+v/Y$) we would find a crossover value of 33% instead of the 1.5% found between white eye and yellow body. The phenotypes of the F_1 females ($y+/+v$) will be wild or normal for both characters, while the males will have red eyes with yellow bodies ($y+/Y$).

The F_2 from such a cross is shown in Table 8-5, for a population of 1000 males and 1000 females.

Table 8-5. Linkage Values in F_2 Population of 2000 from $F_1-y+/+v \text{ } \varnothing$ and $y+/Y \text{ } \sigma$

\varnothing gametes \varnothing gametes		$y+$	y
Parental type	$y+$ 33.5%	$y+/y+$ yellow body red eye 335	$y+/Y$ yellow body red eye 335
Parental type	$+v$ 33.5%	$+v/y+$ gray body red eye 335	$+v/Y$ gray body vermilion eye 335
Crossover type	$++$ 16.5%	$++/y+$ gray body red eye 165	$++/Y$ gray body red eye 165
Crossover type	yv 16.5%	$yv/y+$ yellow body red eye 165	yv/Y yellow body vermilion eye 165

From Table 8-5 it is evident that we can study the linkage value if we consider only the males where 33% of crossover-type flies were found. Since the males are hemizygous for all genes in the X chromosome, the F_2 male phenotypes are exactly like the gametes produced by the F_1 female fly. Thus the X chromosome is highly advantageous for studying linkage. It is possible to determine the linkage of genes without making a testcross.

The crossing-over between yellow body and vermilion is much greater (33%) than for yellow body and white eye (1.5%). This is readily explained by the fact that the genes for yellow and vermilion are much farther apart in the chromosome than yellow and white.

NO CROSSING-OVER IN DROSOPHILA MALES

In the previous experiments, the F_1 female was always the one tested for the production of the different kinds of gametes. In the first example the F_1 female of the genotype $yv/++$ produced four types of gametes, but in different proportions. The F_1 males yv/Y produced only two kinds of gametes yv and Y for the X and Y chromosomes, respectively. It is obvious the male cannot produce any crossover types, since he has only one X chromosome. He is hemizygous for all genes in the X chromosome. The Y chromosome contains no homologues of the genes in the X chromosome, neither recessive nor dominant. The Y chromosome is a blank for all such genes. Because of this fact, we can study the linkage of genes in the X chromosome without the extra labor of making a testcross.

It is easily understood why there can be no crossing-over between the X and Y chromosomes of *Drosophila* males. What is puzzling is why there is never any crossing-over in *Drosophila* males for genes located in the autosomes, where the male has two chromosomes, as do the females. This non-

Table 8-6. Parents, F_1 , and F_2 Showing Segregation of Two Genes in Chromosome 2 of *Drosophila* (data of Bridges)

P		
$+a/+a$ (arc) \times $b+/b+$ (black)		
F ₁		
$+a/b+$	(wild males and females)	
F ₂		
F ₂ Phenotype	Observed	Expected 2:1:1:0
Wild type	923	855
Arc	387	428
Black	401	428
Black arc	0	0

crossing-over in males was discovered by Calvin B. Bridges, one of the distinguished geneticists who received his training with Morgan in the laboratory at Columbia University. Bridges was studying the linkage of black body and a wing character called arc. He mated an arc female with another autosomal mutant, a black-bodied male. Genes for both of these characters are in chromosome 2. In the F_2 he obtained the results shown in Table 8-6. With no crossing-over in males, the results expected are as in Table 8-7. This

Table 8-7. F_2 Phenotypes of Two Autosomal Characters in *Drosophila* with No Crossing-over in the Male

♂ gametes ♀ gametes	+ a	b +
Parental + a	+ a/+ a arc	+ a/b + wild
Parental b +	b +/+ a wild	b +/b + black
Crossover + +	+ +/+ a wild	+ +/b + wild
Crossover b a	b a/+ a arc	b a/b + black

repulsion

table shows that the phenotypes resulting from the parental type gametes in the female gave a ratio of 2 wild : 1 black : 1 arc, as did the phenotypes from the crossover types. Consequently the total result is a 2:1:1 ratio regardless of the amount of crossing-over. As the crossover ratio increases, a greater proportion of the F_2 will be found in the bottom half of the table, which has the *same proportion* as the top half. Thus, the crossover percentage can be ignored. For two genes in the same chromosome entering in the repulsion phase, a ratio of 2 wild : 1 recessive a : 1 recessive b will always be obtained. The double recessive never will be recovered from such a mating. This applies only to the repulsion phase. Results from the coupling phase would be quite different, as is shown in Table 8-8. Here the crossover percentage must be considered. The gene arc is located in the chromosome 2 map at

a distance of 99.5 from the end, while black is at a distance of 48.5. Subtracting, we get a crossover value of 51% for black and arc.

CROSSOVER VALUE CANNOT EXCEED 50 PER CENT

The crossover value for black and arc is approximately the same as for random assortment (50%). With random assortment of gametes, each would be produced in one fourth of the cases, $\frac{1}{4} ba$, $\frac{1}{4} ++$, $\frac{1}{4} b+$ and $\frac{1}{4} +a$. The recombinations would appear in one half of the cases, or 50%. For random assortment the recombination value is always 50%. The value for black and arc was obtained by subtracting the map value of black from that of arc, or 51%. If this were true, it would mean that the recombination classes would be more frequent than the parental types. It would be contrary to all we have learned about the reason for linkage being the proximity of the

Table 8-8. Parents, F_1 , and F_2 of Cross Between Wild ($++/++$) and Black Arc (ba/ba) in *Drosophila*

P

$++/++ \times ba/ba$

F₁

$++/ba$ wild phenotype

F₂

<div style="display: flex; align-items: center; justify-content: center;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">♀ gametes</div> <div style="margin: 0 10px;">↘</div> <div>♂ gametes</div> </div>	$\frac{1}{2} ba$	$\frac{1}{2} ++$	
$\frac{1}{4} ++$	$\frac{1}{8} ++/ba$ wild	$\frac{1}{8} ++/++$ wild	<i>compling</i>
$\frac{1}{4} ba$	$\frac{1}{8} ba/ba$ black arc	$\frac{1}{8} ba/++$ wild	
$\frac{1}{4} +a$	$\frac{1}{8} +a/ba$ arc	$\frac{1}{8} +a/++$ wild	
$\frac{1}{4} b+$	$\frac{1}{8} b+/ba$ black	$\frac{1}{8} b+/++$ wild	

compling

genes in the physical chromosome. Actually, the genes for black and arc have been shown to be in the same chromosome, by studying the linkage values with other genes situated between them. This will be discussed on p. 119 under chromosome mapping. For the present it is enough to know that the recombination value for black and arc is approximately 50%, i.e., random assortment. The F_1 gametes and the F_2 phenotypes are given in Table 8-8 for the mating in the coupling phase.

Table 8-8 shows the following phenotypes: $\frac{5}{8}$ wild, $\frac{1}{8}$ black, $\frac{1}{8}$ arc, and $\frac{1}{8}$ black arc. This is quite different from the mating with the same two genes, but in the repulsion phase. It is also different from the segregation of two genes assorting at random where a 9:3:3:1 ratio would be expected. Although the genes are located so far apart that a random distribution occurs in the formation of the female gamete, there are only two kinds of gametes formed by the male (the parental class). This gives the unusual distribution shown in Table 8-8, because of the absence of crossing-over in the male.

LINKAGE VALUES INVOLVING THREE GENES

Valuable information on linkage relations can be obtained when the cross involves three genes in the same chromosome. Suppose a stock with three linked genes ABC is crossed with the recessive abc , and the F_1 is then testcrossed. This can be diagramed as follows:

$$A B C / a b c \times a b c / a b c$$

It is evident that we can obtain from the F_1 the gametes shown in Table 8-9.

Table 8-9. All Possible Gametes Produced by an F_1 Heterozygous for Three Linked Genes in Coupling Phase

A	B	C	} parental types
a	b	c	
A	b	c	} crossover region 1
a	B	C	
A	B	c	} crossover region 2
a	b	C	
A	b	C	} crossover regions 1 and 2
a	B	c	

In addition to the single crossovers between A and B and between B and C , there occurs in this testcross the double crossover class AbC and aBc . Let us examine an illustration from the actual hybrid involving three different endosperm characters in maize, C , Sh , and wx .

The linkage between c and sh was the first discovered in corn, and it was known as linkage group 1 until it was determined that this group was associated with chromosome 9 (Fig. 8-3). The data are from an experiment of Hutchinson (1922), who discovered the $c sh$ linkage. The cross made by him is shown at the bottom of page 114.

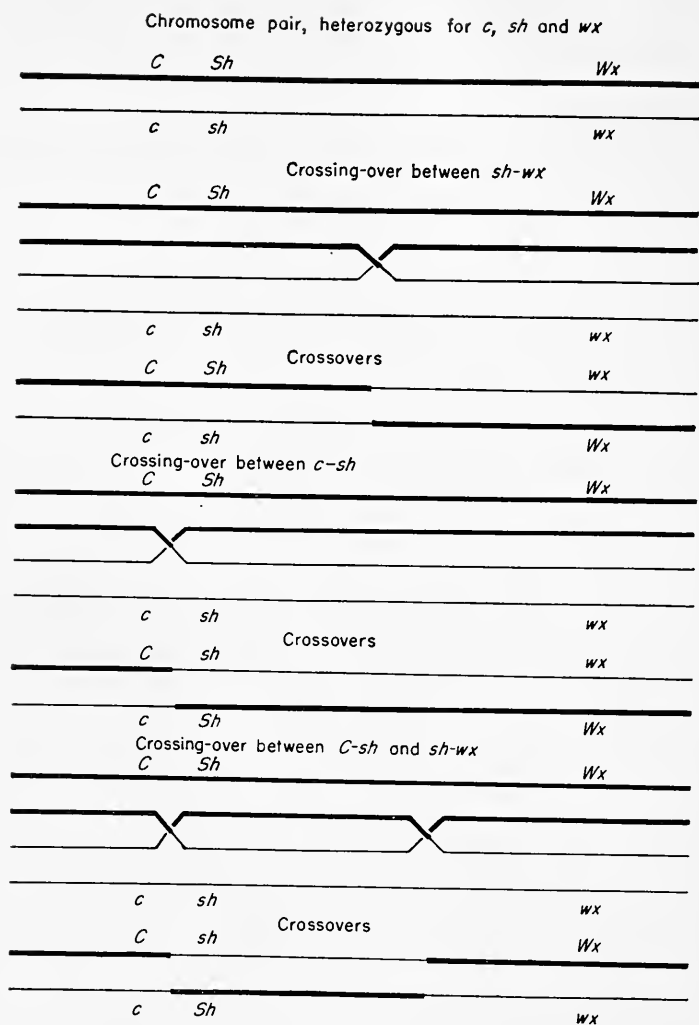
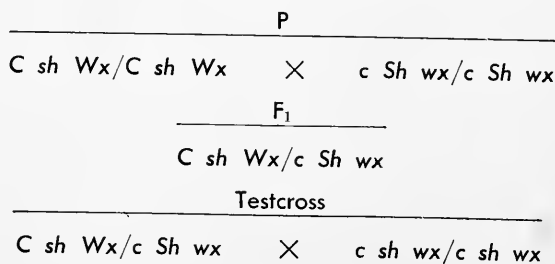


FIG. 8-3 Diagram of chromosome 9 in maize showing relative positions of the genes *c sh* and *wx*. Note single break between *sh* and *wx*, one between *c* and *sh*, and a double crossover involving all three genes.



Since the gamete from the multiple recessive used in making the testcross does not alter the phenotypic expression of any of the gametes contributed by the F_1 female parent, only those from the F_1 are illustrated in Table 8-10.

Table 8-10. Parental and Crossover Gametes Produced by $C\ sh\ Wx/c\ Sh\ wx$ F_1 When Testcrossed

	F_1 Gametes (Also Phenotypes)	% Crossing Over
Parental	$C\ sh\ Wx \dots 2538$ $c\ Sh\ wx \dots 2708$ } ... 5246	
C.O. Region 1	$C\ Sh\ wx \dots 116$ $c\ sh\ Wx \dots 113$ } ... 229	$3.4 + 0.1 = 3.5$
C.O. Region 2	$C\ sh\ wx \dots 601$ $c\ Sh\ Wx \dots 626$ } ... 1227	$18.3 + 0.1 = 18.4$
C.O. Region 1 and 2	$C\ Sh\ Wx \dots 4$ $c\ sh\ wx \dots 2$ } ... 6	0.1
	Total 6708	

Table 8-10 reveals the parental types, the crossover types for region 1 (c and sh), region 2 (sh and wx), and a combination of regions 1 and 2 (c and sh) plus (sh and wx). These data are genetic phenomena determined from breeding behavior alone and not from any visible alteration of the chromosomes. The class of crossovers at both regions 1 and 2 is represented by only six seeds, with but 0.1% double crossing-over. This figure must be added to the values observed for regions 1 and 2 to give the total percentages of crossing-over for regions 1 and 2.

OBSERVED DOUBLE CROSSOVERS LESS THAN EXPECTED: INTERFERENCE AND COINCIDENCE

If the two crossover percentages for regions 1 and 2 were independent of each other, the double crossover value could be obtained by multiplying the crossover per cent for region 1 by that of region 2. If we make such a calculation we get the following: $.035 \times .184 = .0064$ or .6%. The actual D.C.O. found was a .1%, only one sixth of the amount expected on the basis of independence of the two percentages, 3.5% and 18.4%.

In practically all experiments involving linkage of three or more genes, it has been found that crossing-over at one region of the chromosome interferes with crossing-over at other regions. It is as though there were recombinations of small blocks of chromosome rather than of single genes. Consequently, cross-

ing-over at one locus inhibits to some extent crossing-over at adjacent regions. This phenomenon is known as *interference*. The amount of interference may vary in different regions of the same chromosome or between chromosomes. For genes located rather close together, it will be greater than for genes located at a greater distance from each other. In fact, if the three genes are very close together, crossing-over in one region may preclude crossing-over in the second region.

The strength of interference can be stated in another way. This is expressed by a formula obtained by dividing the actual frequency of double crossovers by the expected frequency. To return to the previous illustration of the *c sh wx* linkage, the *coefficient of coincidence* (c.c.) is obtained as follows:

$$\text{c.c.} = \frac{\text{actual double crossover \% (.1)}}{\text{expected double crossover \% (.6)}} = .17$$

The lower the figure, the greater the amount of interference in crossing-over between two regions in the same chromosome.

CROSSING-OVER IN THE FOUR-STRAND STAGE

Ever since crossing-over was first observed, geneticists wondered when it took place. Evidently it happens at meiosis, but is it during the two-strand stage or after the two chromosomes have divided into four chromatids? In most higher plants and animals, it is not possible to identify all the products of a single meiosis. Consequently in maize, for example, crossing-over could occur in either the two-strand or four-strand stage. Hence it is not possible, in maize, to determine the exact timing. This emphasizes the fact that genetic material must be chosen with extreme care to shed light upon the problem under consideration. Although maize is admirable for studying linkage, it will not provide the answer to this particular problem.

CROSSING-OVER IN FOUR-STRAND STAGE IN NEUROSPORA

The advantages of *Neurospora* as a genetic tool have been mentioned before. One of the greatest is that all products of a single meiosis can be observed. Since this is so, we can tell positively whether crossing-over occurs in the four-strand or in the two-strand stage. If only parental types, or only crossover types, were to be found in a single ascus, then we could be certain that crossing-over had occurred in the two-strand stage. However, if *both* the crossover types *and* the parental types are found in the eight ascospores of a single ascus, then we can be sure that crossing-over occurs in the four-strand stage. Actually, it happens as in the second example (Fig. 8-4).

Excellent photographs by David Stadler of *Neurospora* ascospores (Fig.

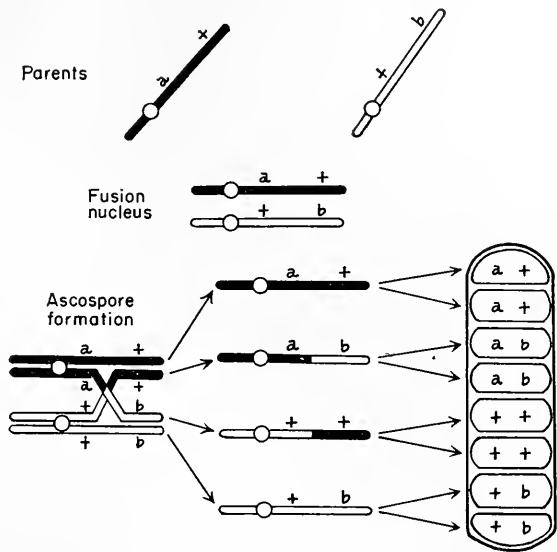
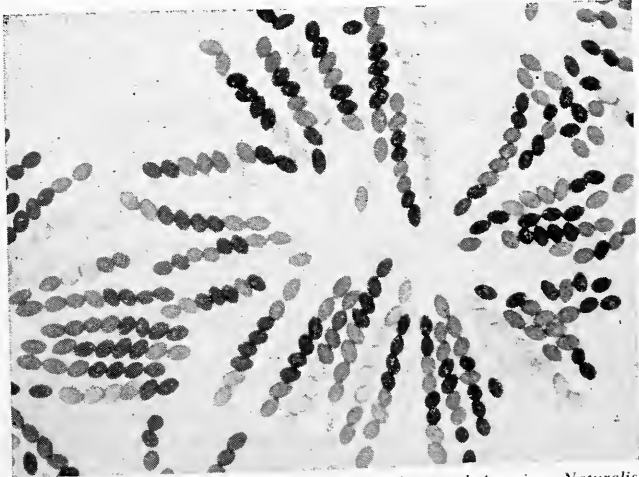


FIG. 8-4 Schematic representation of crossing-over in four-strand stage in *Neurospora*. The four types of spores observed would not be possible if crossing-over had occurred in the two-strand stage between chromosomes, instead of between chromatids.

1-5) and by Lindsay Olive of *Sordaria* ascospores (Fig. 8-5) demonstrate conclusively that crossing-over takes place in the four-strand stage. If it happened in the two-strand stage, the distribution of ascospore phenotypes would always be 4 and 4. The only way one can obtain a 2-2-2-2 distribution of



Courtesy of Lindsay Olive; and American Naturalist

FIG. 8-5 Four-strand crossing-over in *Sordaria* gives asci as shown. A distribution of 2-2-2-2 in ascus, or 2-4-2, demonstrates that crossing-over took place in the four-strand stage.

ascospores in the ascus is for four strands to be present at the time of segregation of the chromosomes (see diagram in Fig. 8-4). A two-strand crossing-over between genes *a* and *b* would give *either* parental *or* crossover types, but not both in the same ascus, as is customarily found in *Neurospora* and in *Sordaria*, in which four-strand crossing-over is established. There is every reason to believe it is common to other organisms.

CROSSING-OVER IN FOUR-STRAND STAGE IN *DROSOPHILA*

It has been demonstrated by experiments with attached X chromosomes in *Drosophila* that crossing-over takes place in the four-strand stage (Fig. 8-6). In an attached X female with the allele for vermillion in one X

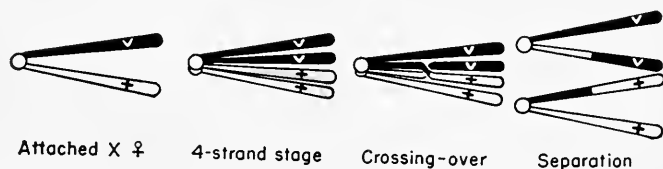


FIG. 8-6 Attached X female in *Drosophila* with *v* in one arm and *+* in the other. Recovery of vermillion flies demonstrates conclusively that crossing-over takes place as illustrated.

chromosome and dominant allele (*+*) in the other, it has been found that such a female mated to a vermillion male will occasionally produce vermillion offspring. Such an unusual F_1 fly must have the vermillion allele (*v*) on both arms of the attached X chromosome. If crossing-over were in the two-strand stage, there would be no possible way to get a chromosome with the *v* on each arm. A crossover would simply change the arm location of the *+* and the *v*. They would both be there, and the wild phenotype would result, owing to the dominance of the *+* allele. If crossing-over occurs in the four-strand stage, however, it is possible to secure a new type of attached X chromosome, one with *v* on each arm of the attached X as actually happened (Fig. 8-6).

CHROMOSOME MAPPING: LINKAGE MAPS

In the second decade of the present century, geneticists were particularly active in studying the inheritance of many characters in plants and animals. Some of the mutants showed linkages of various strengths, and different characters were put into different linkage groups. The groups never exceeded the number of chromosome pairs of the organism. Following Morgan's explanation of linkage and the linear order of genes in 1911, the reason for the association of the different characters in linkage groups was obvious. A

number of characters in *Drosophila* (e.g., white eye, yellow body, and Bar eye) all showed linkage with each other and also the typical sex-linked inheritance characteristic of white eye. It was safely concluded that this list of genes was in the X chromosome of the XY pair.

Two other large linkage groups were soon established in *Drosophila*. Genes located in each of these two groups showed random assortment between genes in the others, but linkage among themselves. They are located in two autosomes, No. 2 and No. 3, and are not sex-linked.

A third and smaller autosomal linkage group was found to be located in chromosome 4, that pair of dotlike chromosomes, by far the smallest in *Drosophila*.

In maize, also, linkage groups were being established. The first of these was the *c sh wx* group, which for a time was known as linkage group No. 1 in corn. McClintock's brilliant work in the late 1920's and early 1930's identified the ten pairs of chromosomes morphologically and associated them with the different linkage groups. She found that the first linkage group was actually in chromosome 9, the next to the shortest in maize.

METHOD OF CONSTRUCTING LINKAGE MAPS

Once a linkage has been determined between two genes, a linkage group is established. If an additional gene shows linkage with one of the first two, it then joins the family of genes in that chromosome. For example, the genes *c* and *sh* were first found to be linked with a crossover percentage of 3.3. This was the beginning of a linkage map.

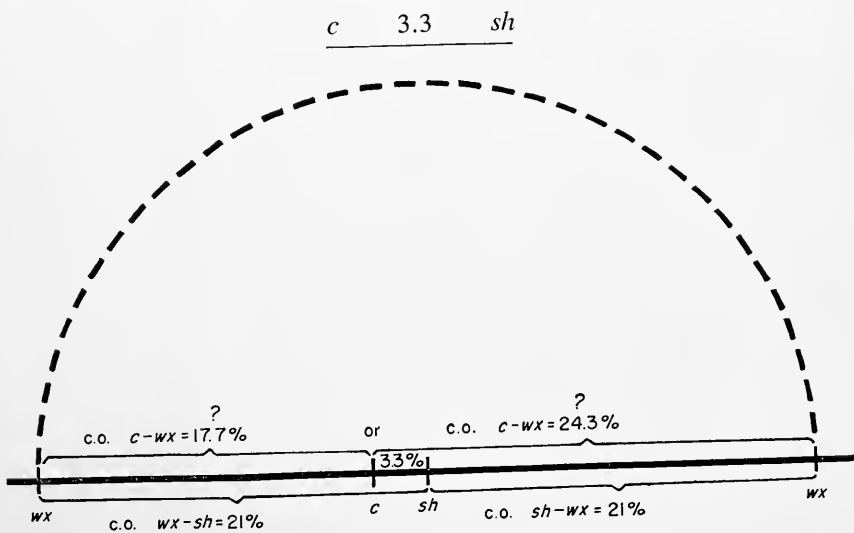


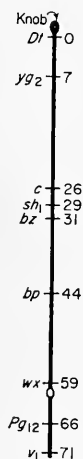
FIG. 8-7 Placement of a third gene (*wx*) in a linkage map of chromosome 9 in maize. "Rainbow" indicates *wx* may be to right or left of *sh*.

Another gene, waxy (*wx*), affecting starch composition, was shown to be linked with shrunken (*sh*), with a crossover percentage of 21. Consequently, *wx* should show a linkage with the *c* locus. It could be to the right of the *sh* locus or to the left (Fig. 8-7).

If the first order is correct, then the linkage between *c* and *wx* should be approximately 24, whereas, if the second order were correct, the linkage of *wx* and *c* should be about 18. Results of many trials give a value of 26 for crossing-over between *c* and *wx*, showing conclusively that *wx* is to the right of *sh*, the order being *c—sh—wx*.

By a similar process, the linkage map for chromosome 9 has been extended in both directions, to the left of *c* and to the right of *wx*. The directions left and right are purely arbitrary and bear no relationship to the morphology of the chromosome. Actually, all three genes are in the short arm of the chromosome with the *wx* locus being nearest the centromere. Also, genes have been added between *sh* and *wx*, and by so doing the linkage value for *sh—wx* has been increased. The present linkage map for chromosome 9 is shown in Fig. 8-8, beginning with the terminal knob on the end of the short arm of the chromosome.

FIG. 8-8 Linkage map for chromosome 9 in maize (first linkage group established in maize). The names of genes represented by symbols can be found in Appendix A.



It will be observed that the map distance from *sh1* (29) to *wx* (59) is now 30 units, in contrast to the 21 indicated in the earlier map between *sh1* and *wx*. Part of this increase may be accounted for by more extensive data since the value of 21 was found. Most of it can be explained by the addition of two genes *bz* (bronze plant and aleurone color) and *bp* (brown pericarp) between *sh* and *wx*. Before these two genes were discovered, the region between *sh* and *wx* apparently could cross over at will without being detected.

With the two additional genes *bz* and *bp* to serve as markers, additional cross-overs could be detected. Hence the map distance (based on crossover percentages) was increased.

It is now possible to detect additional crossover types, as shown in Table 8-11.

Without the presence of genes *bz* and *bp*, it was possible to detect only two crossover types, *sh Wx* and *Sh wx*, in addition to the two parental types. Consequently, the inclusion of *bz* and *bp* in the four-point linkage test has enabled us to explore the formerly unknown space between the two end points *sh* and *wx*. By establishing crossovers here we have "increased" the map distance between *sh* and *wx* from 21 to 30. By similar experiments, all chromosomes of maize, *Drosophila*, and several other organisms have been carefully mapped. The longest chromosome in maize has a map distance of 161, built up by studying crossover values of two, three, or four genes at a time. This linkage

Table 8-11. Crossover Gametes Produced in Chromosome 9 in Maize with Four Heterozygous Genes

Genes			Crossover region
<i>sh</i>	<i>Bz</i>	<i>Bp</i>	<i>Wx</i> 1
<i>Sh</i>	<i>bz</i>	<i>bp</i>	<i>wx</i> 1
<i>sh</i>	<i>bz</i>	<i>Bp</i>	<i>Wx</i> 2
<i>Sh</i>	<i>Bz</i>	<i>bp</i>	<i>wx</i> 2
<i>sh</i>	<i>bz</i>	<i>bp</i>	<i>Wx</i> 3
<i>Sh</i>	<i>Bz</i>	<i>Bp</i>	<i>wx</i> 3
<i>sh</i>	<i>Bz</i>	<i>bp</i>	<i>wx</i> 1 and 2
<i>Sh</i>	<i>bz</i>	<i>Bp</i>	<i>Wx</i> 1 and 2
<i>sh</i>	<i>bz</i>	<i>Bp</i>	<i>wx</i> 2 and 3
<i>Sh</i>	<i>Bz</i>	<i>bp</i>	<i>Wx</i> 2 and 3
<i>sh</i>	<i>Bz</i>	<i>Bp</i>	<i>wx</i> 1 and 3
<i>Sh</i>	<i>bz</i>	<i>bp</i>	<i>Wx</i> 1 and 3
<i>sh</i>	<i>Bz</i>	<i>bp</i>	<i>Wx</i> 1, 2, and 3
<i>Sh</i>	<i>bz</i>	<i>Bp</i>	<i>wx</i> 1, 2, and 3

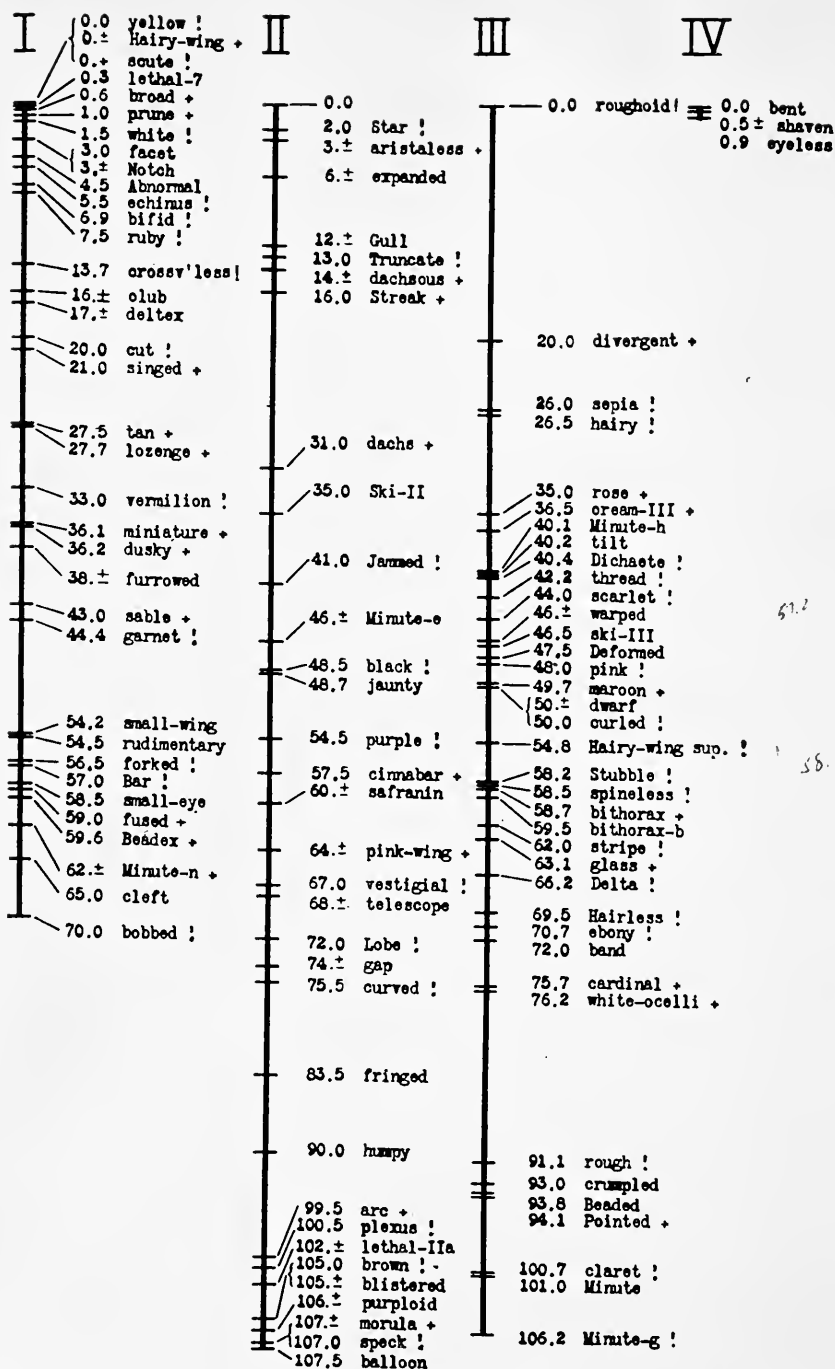
map of 161 does not mean that the two characters on the ends of the chromosome would show 161% of crossovers. It would be impossible to have any value greater than 50%. It has been pointed out before that the crossover percentage can never exceed 50, since such a figure means that crossover and parental types are recovered with equal frequency. This is random assortment and applies equally well to genes not located in the same chromosome, as to those located far apart in the same chromosome.

In chromosome 1 the genes *bm*₂ and *br*₁ show 46% of recombinations. They have a difference in map distance of 81 (161 less 80). Also the genes *br*₁ and *bm*₂ show a recombination of 47%. The difference in map distance is 53 (80 less 27). Thus it would be possible to have three genes in chromosome 1 showing virtually no linkage (approximately 50% recombinations). Their presence in the same chromosome was not established until some of the unexplored territory between them was studied genetically. As soon as linkages in between became known, it was possible to join the outermost genes *ts*₂ and *bm*₂ with the midpoint *br*₁, and consequently with each other.

Linkage values for genes in other organisms have been established by the method outlined for maize. Typical linkage maps are given for *Drosophila*, maize, and mice in Figs. 8-9, 8-10, and 8-11.

CONCLUDING REMARKS

In this chapter we have examined inheritance of genes showing nonrandom assortment in an *F*₂ or a testcross. Genes entering a hybrid in a



From Morgan; Courtesy of Yale University Press

FIG. 8-9 Linkage map for *Drosophila*.

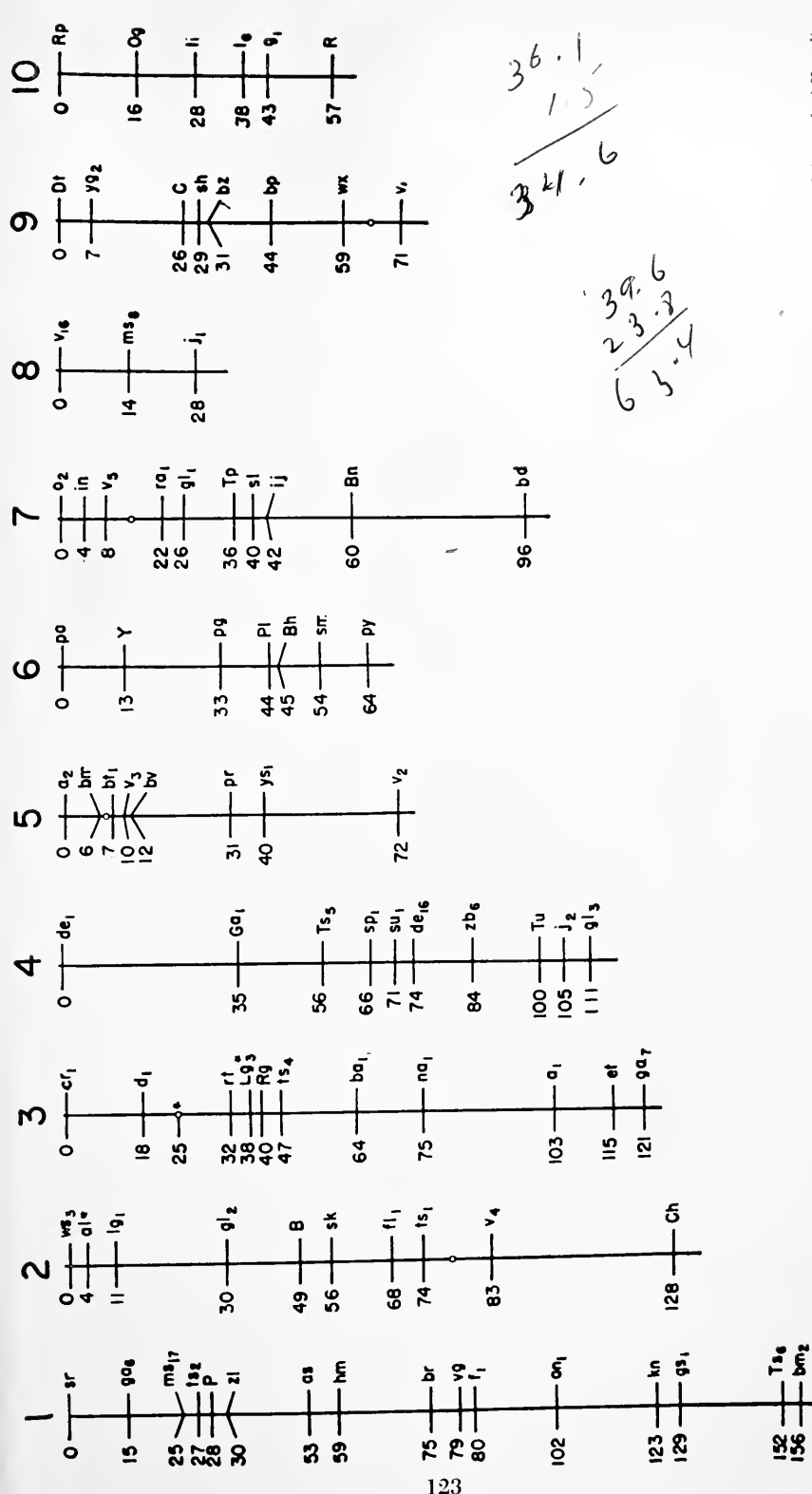
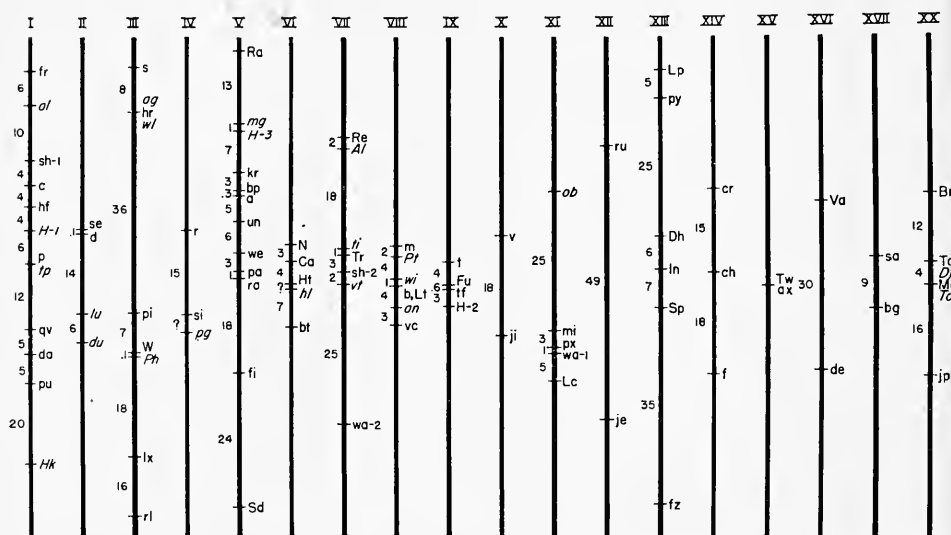


FIG. 8-10 Linkage map of maize.

certain combination tend to segregate from the hybrid in such a manner that the parental types are recovered more frequently than the recombinations. For example, in the F_1 hybrid between AB/AB and ab/ab , the parental types AB and ab are recovered more frequently than the recombinant types Ab and aB . The deviation from a 1:1 ratio for crossovers and parental types varies, depending upon how close the two genes are in the chromosome. In *Drosophila*, recombinations between white eye (w) and yellow body (y) occur in only about 1.5% of the offspring, while recombinations between yellow



Courtesy of Green and Dickie; and Journal of Heredity.

FIG. 8-11 Linkage map of mouse, the best-mapped mammal. The names of genes represented by symbols can be found in Appendix B.

body (y) and vermilion eye (v) occur in 33% of the gametes formed. All genes in *Drosophila* are assorted in four linkage groups corresponding to the four pairs of chromosomes. In maize there are ten linkage groups associated with the ten morphologically distinct chromosomes. Mice have 20 linkage groups in the 20 chromosome pairs.

Crossover values may vary from zero to 50%. The latter value is the one for random assortment. It has been adequately demonstrated in *Drosophila*, *Neurospora*, and *Sordaria* that crossing-over occurs in the four-strand stage. Most likely, this applies to other organisms as well. When additional genetic information accumulates for various organisms, linkage groups are established. The linkage groups never exceed the haploid number of chromosomes of the species being studied.

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PROBLEMS

8-1. Define the following terms:

chiasma (pl. chiasmata)	hemizygous
chromatid	interference
chromosome mapping	linkage map of <i>Drosophila</i>
coefficient of coincidence	linkage map of maize
coupling	linkage map of mice
crossover	non-random assortment
double crossovers	recombination
factors	repulsion
four-strand crossing-over	

8-2. Identify the following scientists, giving a major contribution of each, with an approximate date.

Bateson, William	McClintock, Barbara
Bridges, Calvin B.	Morgan, T. H.
Hutchinson, C. B.	Olive, L. S.

8-3. Make a mating between a yellow-white-minature (*y w mn*) female and a wild male. The genes are in the right order. The CO between *y* and *w* is 1.5%; between *w* and *mn* 35%. Assume a coefficient of coincidence of 0.5. In an F_2 population of 1000 males and 1000 females, how many of each phenotype would be obtained?

8-4. A cross was made between a *C/C sh/sh Wx/Wx* stock of maize and one with the genotype *c/c Sh/Sh wx/wx*. Give the constitution of the gametes produced by the parents, and also the genotype of the F_1 . The *C* stands for color, the *Sh* is plump, and the *sh* is shrunken. The *Wx* has normal starch, while the *wx* has waxy which stains red with iodine. What would the F_1 seeds look like?

A testcross of the F_1 gave the following phenotypes:-

<i>C</i>	<i>sh</i>	<i>Wx</i>	2777
<i>c</i>	<i>Sh</i>	<i>wx</i>	2708
<i>C</i>	<i>Sh</i>	<i>wx</i>	116
<i>c</i>	<i>sh</i>	<i>Wx</i>	123

<i>C</i>	<i>sh</i>	<i>wx</i>	643
<i>c</i>	<i>Sh</i>	<i>Wx</i>	626
<i>C</i>	<i>Sh</i>	<i>Wx</i>	4
<i>c</i>	<i>sh</i>	<i>wx</i>	3
			<hr/> 7000

Calculate the crossing-over between *C* and *sh*, also between *Sh* and *wx*. Calculate the per cent of double crossovers and the coefficient of coincidence. Make a linkage map of chromosome 9 showing the genes in the chromosome approximately to scale.

- 8-5. In *Drosophila* two autosomal genes are in the same chromosome. In a cross between a black stock and one having a wing variant arc, the parents would have the following genetic constitution:

$$arc+/arc+ \text{ and } +b/+b$$

Give the genotype of the F_1 and show by a checkerboard the types of individuals that would be expected in the F_2 . What ratio would you expect of the following types? (Assume a population of 1000.)

wild	black
arc	arc black

- 8-6. In chromosome 3 of *Drosophila* curled (*cu*) and ebony (*e*) are approximately 20 units apart. Give the genotypes of parents and the F_1 . In an F_2 of flies involving these two genes in the coupling phase, how many of each phenotype would be expected in a population of 1000 flies?
- 8-7. In maize three genes are linked in one chromosome. Assume one parent is dominant for all three genes, the other recessive. In a testcross the following numbers were obtained (phenotypes only will be given).

	<i>A</i>	<i>B</i>	<i>C</i>	225
	<i>a</i>	<i>b</i>	<i>c</i>	245
140	<i>a</i>	<i>B</i>	<i>c</i>	98
	<i>A</i>	<i>b</i>	<i>C</i>	102
	<i>A</i>	<i>B</i>	<i>c</i>	144
11	<i>a</i>	<i>b</i>	<i>C</i>	156
	<i>a</i>	<i>B</i>	<i>C</i>	14
1	<i>A</i>	<i>b</i>	<i>c</i>	16
Total				<hr/> 1000

Arrange the genes in the correct linear order. What is the crossing-over percentage for regions 1 and region 2 of the chromosome arranged in the proper linear order? Is there interference? What is the coefficient of coincidence?

- 8-8. A cross was made between two different genetic strains in *Neurospora*. Write the genotypes of the parental strains, one being recessive for gene *a*, the other being recessive for gene *b*. Assume gene *a* is so near the centromere that no crossing-over occurs between it and the centromere. Gene *b* is in the homologous chromosome 20 crossover units away. In 1000 ascospores how many of each type would be obtained?
- 8-9. The character scarlet eye (*st/st*) and ebony body (*e/e*) are located in

chromosome 3 of *Drosophila*, approximately 27 crossover units apart. A cross is made between scarlet and ebony.

Give complete genotype of the parents and F_1 . List the phenotype of F_1 as well as phenotypes of F_2 . Assume an F_2 population of 1000.

- 8-10. Demonstrate conclusively (by a diagram) that crossing-over occurs in the four-strand stage of *Neurospora*. As proof, show consequences of crossing-over in the two-strand stage.
- 8-11. Demonstrate conclusively (by a diagram) that crossing-over occurs in the four-strand stage of *Drosophila*. Show consequences of crossing-over in the two-strand stage.
- 8-12. In *Drosophila* attached X stocks show unusual genetic behavior of sex-linked characters. If an attached X vermilion female is mated to a white miniature male, what kinds of F_1 progeny will be observed? Show by a Punnett square the results of this mating. Write the genotypes of the parents and the F_1 . What results are expected in the F_2 ?

Note: The attached X female also has a Y chromosome (XXY). Flies of the genotypes XXX and YY are non-viable.

Genic Interaction

IN THE preceding chapters we have discussed the genetics of characters whose inheritance is conditioned by a single gene. To be more precise, we should say that the inheritance is determined by a single gene *difference*. For example, the first mutant found in *Drosophila* was a white-eyed male. Morgan learned that this phenotypic expression was conditioned by a single, sex-linked gene located in the X chromosome. However, this white-eyed male had thousands of genes exactly like those in wild individuals with red eyes. It is as though there were a chain of thousands of links and, to produce an effect, the chain need break at only *one link*. All others would remain the same.

The white-eyed male found by Morgan represents a mutation at a specific location (locus) near the end of the X chromosome. There are thousands of sites where mutation may occur. This one just happened to be in chromosome 1, about 1.5 crossover units from the end. The phenotypic expression, white eye, is caused by a *single gene difference*. The other genes for eye color were normal (+) for all loci.

WHITE EYE DUE TO TWO RECESSIVE GENES

In addition to the white eye of *Drosophila*, caused by a single gene at locus 1.5 in chromosome 1 (the X chromosome), a white-eyed phenotype is produced by the interaction of two recessive genes, for brown (*bw*) and for scarlet (*st*), two autosomal mutants in chromosomes 2 and 3, respectively. A fly that is *bw/bw* and has at least one dominant allele for the *st* locus, *st*⁺/—, has a distinctly pinkish brown eye, without the characteristic dark spot of the wild-type red eye. Likewise, a fly that is homozygous recessive for the *st* gene and possesses either one or two dominant alleles, of the brown locus, *bw*⁺/—, has a bright scarlet eye, much brighter and lighter in shade

than the wild-type red eye. It is also without the dark spot or, at the most, has a very faint dark spot.

The double recessive $bw/bw\ st/st$, however, has a white eye phenotypically indistinguishable from the white eye conditioned by the w gene in chromosome 1 (Plate III). Here, then, is the interaction of the recessive genes at two different loci to produce a distinct phenotypic effect. The mechanism of the interaction is not completely understood, but the phenotypic expression is distinct and striking.

The white-eye character illustrates the point that the phenotypic result observed may be a cooperative effort of two genes or may be produced by a single gene difference. Actually, all phenotypic effects represent a "team effort" and not the action of a single gene, even though one allele, or the lack of it, may be primarily responsible for the effect.

COMPLEMENTARY GENES IN SWEET PEAS

The first clear case of complementary gene action was shown by Bateson and Punnett, two British geneticists, in studying the inheritance of color in sweet peas. Actually, they began their work with the sweet pea by a study of the inheritance of pollen shape. They crossed two white types, phenotypically alike in every respect except for the shape of the pollen grains. One had the normal long pollen grains of the species, the other round. While both parental types bore white flowers, the F_1 rather surprisingly had purple flowers. This was a startling discovery, coming as it did within the first decade of the present century.

The investigators satisfactorily explained their results by the assumption of two complementary genes, the presence of at least one dominant allele of each being necessary for the production of any color. The genotypes of the parents were $C/C\ r/r$ and $c/c\ R/R$. The gene r in the homozygous condition prevented color formation, as did the homozygous recessive c/c . The F_1 was $C/c\ R/r$. It had fully colored flowers because of the dominance of C and R over their recessive alleles, and by the complementary nature of the interaction of the two genes C and R . It is as though one gene (C) is responsible for an enzyme necessary to produce a substrate for color formation, while the other gene (R) is responsible for an enzyme that converts the substrate to an anthocyanin, the pigment formed. The genotype $C/C\ r/r$ is colorless because of the lack of an enzyme producing a substrate. The genotype $c/c\ R/R$ lacks color because it does not have another enzyme to convert the substrate to anthocyanin.

The segregation of the F_1 , $C/c\ R/r$, assuming no linkage of these two genes, would give the proportions in the F_2 shown in (Table 9-1).

The class of $\frac{9}{16}\ C/\text{—}\ R/\text{—}$ is the only one capable of producing color. This is because of the presence of both the enzyme for the substrate and of the enzyme for converting it to anthocyanin, giving a 9:7 ratio of colored to white.

Table 9-1. Zygotic Checkerboard Showing F_2 Segregation for Two Complementary Genes in Sweet Peas

<div style="display: inline-block; transform: rotate(-45deg);"> Segregation for C/c R/r Segregation </div>	$\frac{3}{4} C/-$	$\frac{1}{4} c/c$
$\frac{3}{4} R/-$	$\frac{9}{16} C/- \quad R/-$ colored	$\frac{3}{16} c/c \quad R/-$ colorless
$\frac{1}{4} r/r$	$\frac{3}{16} C/- \quad r/r$ colorless	$\frac{1}{16} c/c \quad r/r$ colorless

This experiment of Bateson and Punnett represents the first, and one of the clearest, demonstrations of the complementary nature of genic interaction. As was pointed out in Chapter 6, there is general genic interaction with a host of other "normal" genes, as well as this specific interaction between two specialized complementary genes.

COMPLEMENTARY GENES IN MAIZE

Inheritance similar to the one just described for sweet peas was found by East and Hayes to exist for aleurone color in maize. The aleurone is a single layer of cells just beneath the pericarp of the corn kernel (Fig. 9-1). However, instead of just two complementary genes, maize was found to have three. Two of these, $C/-$ and $R/-$, had the same designation as in sweet peas and their inheritance was identical. A cross of $C/C \ r/r$ by $c/c \ R/R$, both colorless, produced a colored F_1 and nine colored to seven colorless in the F_2 . An additional gene, A , was found by Emerson to be necessary for any anthocyanin production. All of these three genes, A , C , and R , were later shown to be in separate chromosomes, confirming the reason for the independent inheritance observed by East, Hayes, and Emerson.

If we add the independent segregation for A/a to that observed for C and R (see Table 9-1 for sweet peas) we get the results shown in Table 9-2. The $\frac{27}{64}$ in the upper left-hand corner of the progeny represents the only colored kernels, while the other $\frac{37}{64}$ are all colorless. This is because each of

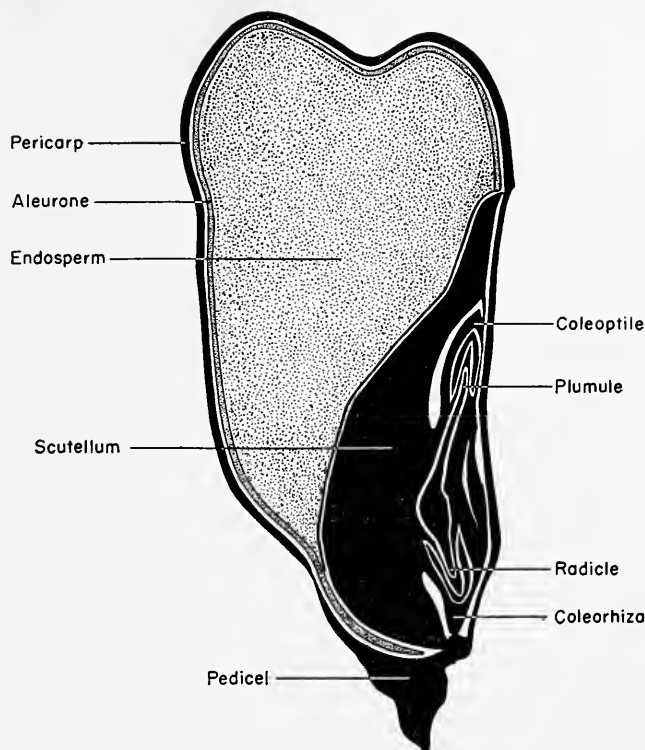


FIG. 9-1 Outline of a corn kernel showing location of color produced by $A/-$ $C/-$ $R/-$ $Pr/-$ in aleurone layer. The yellow or white color $Y/-$ or y/y is in the endosperm, beneath the aleurone layer. Black: $2n$ tissue; stippled: $3n$ tissue.

these classes lacks at least one of the dominant alleles necessary for anthocyanin production. Actually, a fourth gene, labeled A_2 , is necessary for anthocyanin production. A_2 is located in chromosome 5 and shows no linkage with A_1 (chromosome 3), C (chromosome 9), and R (chromosome 10). If all four genes for anthocyanin were heterozygous, an F_1 plant self-pollinated would produce an ear with 81 colored kernels, and 175 not colored, out of a population of 256.

LIMITED PHENOTYPIC EXPRESSION

The genes A , C , and R in maize work together in a complementary fashion to produce color when all three are present. Another gene pr causes the anthocyanin to be red, instead of dark blue or purple, when the dominant gene Pr is present in at least one allele. It is evident that this pair of alleles will be unable to show any phenotypic expression in kernels lacking anthocyanin in the aleurone layer. $Pr/-$ and pr/pr kernels would be pheno-

Table 9-2. Zygotic Checkerboard Showing F_2 Segregation of Three Complementary Genes in Maize

Segregation for C/c R/r				
	A/a Segregation			
$\frac{3}{4} A/-$	$\frac{9}{16} C/- R/-$	$\frac{3}{16} C/- r/r$	$\frac{3}{16} c/c R/-$	$\frac{1}{16} c/c r/r$
	$\frac{27}{64} A/- C/- R/-$	$\frac{9}{64} A/- C/- r/r$	$\frac{9}{64} A/- c/c R/-$	$\frac{3}{64} A/- c/c r/r$
$\frac{1}{4} a/a$	$\frac{9}{64} a/a C/- R/-$	$\frac{3}{64} a/a C/- r/r$	$\frac{3}{64} a/a c/c R/-$	$\frac{1}{64} a/a c/c r/r$
	$\frac{9}{64} a/a C/- R/-$	$\frac{3}{64} a/a C/- r/r$	$\frac{3}{64} a/a c/c R/-$	$\frac{1}{64} a/a c/c r/r$

Table 9-3. F_2 Segregation of R/r and Pr/pr in Maize. Fr/pr Expressed Only in Presence of R .

Pr/pr Segregation	Segregation for R/r	
	$\frac{3}{4} R/-$	$\frac{1}{4} r/r$
$\frac{3}{4} Pr/-$	$\frac{9}{16} R/- Pr/-$ purple	$\frac{3}{16} r/r Pr/-$ colorless
$\frac{1}{4} pr/pr$	$\frac{3}{16} R/- pr/pr$ red	$\frac{1}{16} r/r pr/pr$ colorless

Genic Interaction

typically alike (colorless). If a red stock $A/A\ C/C\ R/R\ pr/pr$ were crossed by a colorless stock, due to the absence of a dominant R ($A/A\ C/C\ r/r\ Pr/Pr$) stock, there would be but two genes segregating. It is not necessary to write the composition for A and C in this case, as they will be A/A and C/C . Here we are concerned, as is always the case in genetics, with genic differences. The two stocks have many more dominant genes in common than A/A and C/C . The segregation for R/r and Pr/pr is shown in Table 9-3.

Here we get a 9:3:4 ratio instead of a 9:3:3:1 because of our inability to distinguish Pr and pr phenotypes in the colorless kernels. (Frontispiece, 7 o'clock.)

Likewise if the gene for yellow endosperm is heterozygous (Y/y) instead of Pr/pr , in addition to R/r , another type of distorted 9:3:3:1 ratio is obtained (Table 9-4). In this case the ratio is twelve colored (all purple) to three yellow to one white. There is no segregation for different colors of anthocyanin because both stocks are homozygous Pr/Pr . (Frontispiece, 5 o'clock.)

Table 9-4. Parents F_1 and F_2 of Cross Between Yellow, Purple, and a Colorless Aleurone White-seeded Stock

P					
A/A	C/C	R/R	Pr/Pr	Y/Y = purple	
X					
A/A	C/C	r/r	Pr/Pr	y/y = white	
F ₁					
R/r		Y/y (only genes segregating)			
F ₂					
Y/y Segregation	Segregation for R/r	$\frac{3}{4} R/-$		$\frac{1}{4} r/r$	
$\frac{3}{4} Y/-$		$\frac{9}{16} R/- \quad Y/-$ colored (purple)		$\frac{3}{16} Y/- \quad r/r$ yellow	
$\frac{1}{4} y/y$		$\frac{3}{16} R/- \quad y/y$ colored (purple)		$\frac{1}{16} r/r \quad y/y$ white	

If all three of the genes heterozygous in the last two ratios are combined in a single segregation, a 9:3:3:1 ratio is obtained. However, three genes are segregating, instead of the usual two that produce a 9:3:3:1 ratio. Since the Y/y segregation can be expressed only in colorless seeds, while the Pr/pr segregation can occur only in colored seeds, it is obvious the genes Pr/pr and Y/y can cause segregations in both colorless and colored seeds. In other words, combining a 9:3:4 ratio and a 12:3:1 ratio results in a 9:3:3:1 segregation (Frontispiece). Such an ear would have nine $Pr/-$ (purple) to three pr/pr (red) to three $Y/-$ (yellow) to one y/y (white). This is shown in the zygotic checkerboard of Table 9-5. It is not necessary to have a separate column for the Pr/pr and the Y/y segregation. The segregation for Pr/pr can occur only in colored kernels $R/-$, while the Y/y segregation can be observed only in colorless r/r . (Frontispiece, 6 o'clock.)

Table 9-5. F_2 Segregation of Three Genes R/r , Pr/pr , and Y/y , Giving a 9:3:3:1 Segregation

Segregation for R/r Pr/pr or Y/y Segregation	Segregation for R/r	
	$\frac{3}{4} R/-$	$\frac{1}{4} r/r$
$\frac{3}{4} Pr/pr$ or Y/y	$\frac{9}{16} R/- Pr/-$ purple	$\frac{3}{16} Y/-$ yellow
$\frac{1}{4} pr/pr$ or y/y	$\frac{3}{16} R/- pr/pr$ red	$\frac{1}{16} r/r y/y$ white

COLOR INHIBITOR IN MAIZE ALEURONE

In contrast to the colorless kernels due to a lack of at least one of the dominant alleles of the three basic color genes, A , C , and R , there is another class of colorless kernels caused by a dominant inhibitor, I . The precise designation should be CI , because the inhibitor is a gene closely linked to C in chromosome 9. Almost no crossing-over is observed between C and I . Suppose we cross a colorless stock of the constitution $A/A CI/CI R/R$ by one that is $a/a C/C R/R$, the F_2 would then be as shown in Table 9-6. In this

Table 9-6. F₂ Segregation of Two Gene Pairs A/a and Cl/C Giving 13:3 Ratio

<div>Segregation for A/a</div> <div>Cl/C Segregation</div>	$\frac{3}{4} A/-$	$\frac{1}{4} a/a$
	$\frac{9}{16} A/- \quad Cl/-$ colorless	$\frac{3}{16} a/a \quad Cl/-$ colorless
$\frac{3}{4} Cl/-$		
$\frac{1}{4} C/C$	$\frac{3}{16} A/- \quad C/C$ colored	$\frac{1}{16} a/a \quad C/C$ colorless

segregation the only colored class will be the lower left with the constitution A/- C/C. This gives a ratio of 13 colorless to 3 colored (Frontispiece).

These are but a few of the many ratios that are possible by manipulation of the genes for aleurone color in maize. A rather complex one would be obtained if an inhibitor stock A/A Cl/Cl R/R were crossed by a colorless stock of the genotype a/a C/C r/r. The F₁ would be colorless (A/a Cl/C R/r). In the F₂ there would be $\frac{9}{64}$ colored with the remaining $\frac{55}{64}$ colorless. The segregation for Pr/pr and Y/y could be added to the one above by means of the zygotic checkerboard. Students may wish to do this as an exercise in multigenic segregation. It is not necessary to include more examples of aleurone segregation in the text, as the basic ones have been given.

It should be pointed out that the science of genetics has moved rapidly in recent years, from the formulation of complicated ratios for a large number of genes segregating, to a more detailed analysis of a few. As a result, our understanding of the way in which a gene produces results has increased markedly.

We believe that the zygotic checkerboard will enable the student to grasp the mechanics more quickly, thus leaving time for an understanding of the physio-chemical nature of gene action. A possible clue to gene action is found in the next section, in which we describe complementary genic interaction in production of high cyanide content in white clover.

CYANIDE PRODUCTION IN WHITE CLOVER

Cyanide production in white clover has a pattern of inheritance very similar to that of aleurone color in maize. Some strains of white clover

have a high cyanide content, others low. High content is dominant, in that a cross of high by low invariably gives an F_1 with high content. If we let a/a = low and A/A = high, a cross between a low and a high could be diagrammed thus:

$$\begin{array}{ll} P & a/a \times A/A \\ F_1 & A/a \text{ high} \\ F_2 & 3 A/- \text{ (high)} : 1 a/a \text{ (low)} \end{array}$$

It should be pointed out that high cyanide strains are not toxic to animals but, on the contrary, are higher in nutritive value.

The monogenic segregation for $3 A/- : 1 a/a$ is similar to the aleurone color in corn when only one gene is segregating, i.e., $3 A/- : 1 a/a$.

However, it has been shown that the inheritance of cyanide production resembles aleurone color in corn in another respect. More than one gene for high cyanide has been found. The second gene might be termed B/B for high and b/b for low, and the B/b gene alone would give a 3:1 segregation.

In some instances, however, two strains low in cyanide have given an F_1

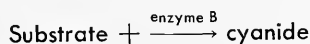
Table 9-7. Inheritance of High Cyanide in White Clover

P			
A/A (low because of b/b)	b/b	\times	a/a B/B (low because of a/a)
F ₁			
A/a	B/b = high		
F ₂			
B/b Segregation	Segregation for A/a	$\frac{3}{4} A/-$	$\frac{1}{4} a/a$
$\frac{3}{4} B/-$		$\frac{9}{16} A/- B/-$ high	$\frac{3}{16} a/a B/-$ low
$\frac{1}{4} b/b$		$\frac{3}{16} A/- b/b$ low	$\frac{1}{16} a/a b/b$ low

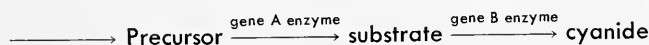
that is high. Here we have complementary genes, again similar to the *A* and *C* genes for aleurone color in corn. The inheritance of high cyanide is shown in Table 9-7. Likewise, we encounter the 9:7 ratio for two complementary genes segregating. The seven class exists because of our inability to distinguish the two recessive genotypes *a/a* and *b/b* when present separately or in combination *a/a b/b*.

The cyanide inheritance is useful in telling us something of how the genes operate to produce their results. It is known that an enzyme is necessary that, when acting upon a substrate, produces hydrocyanic acid (HCN). The substrate is known as cyanogenic glucoside.

It is known that one of the low cyanide strains possesses very little enzyme. If we let strains *A/A b/b* represent the low in enzyme we would get very little, if any, cyanide—even with abundant substrate. With the composition *B/B* or *B/b*, however, the enzyme would be supplied, and we would get a reaction like this:



It is also probable that gene *A* governs an enzyme that produces the substrate from the precursor. In this case we could diagram the whole reaction as follows:



This illustration is particularly pertinent because it gives an indication of how the genes operate to produce their results. Our best hypothesis as to how genes act is that each gene controls an enzyme which, acting at the proper time, produces a certain chemical substance. We will go into this in more detail in Chapter 18.

INHERITANCE OF COMB SHAPE IN POULTRY

As an example of interaction of genes in animals to give a di-hybrid ratio, let us consider the inheritance of comb shape in fowl. Many breeds of poultry have the erect single comb, familiar to everyone. Some breeds have a much flattened, elongated comb, broad at the front and tapering to a point at the back. This is known as the rose comb. Others have a narrower, higher, ridged feature called the pea comb. Crosses between rose and single show that rose is dominant, and in the F_2 a 3:1 ratio is obtained. Also, the pea comb is dominant over the single comb and in the F_2 gives a 3:1 ratio.

However, when a cross is made between a rose and a pea comb an entirely new type of comb arises, similar in shape to a walnut meat, hence the name walnut. Research has shown that two genes are involved in this cross, as we might have suspected, since both rose and pea comb give a 3:1 ratio in crosses with

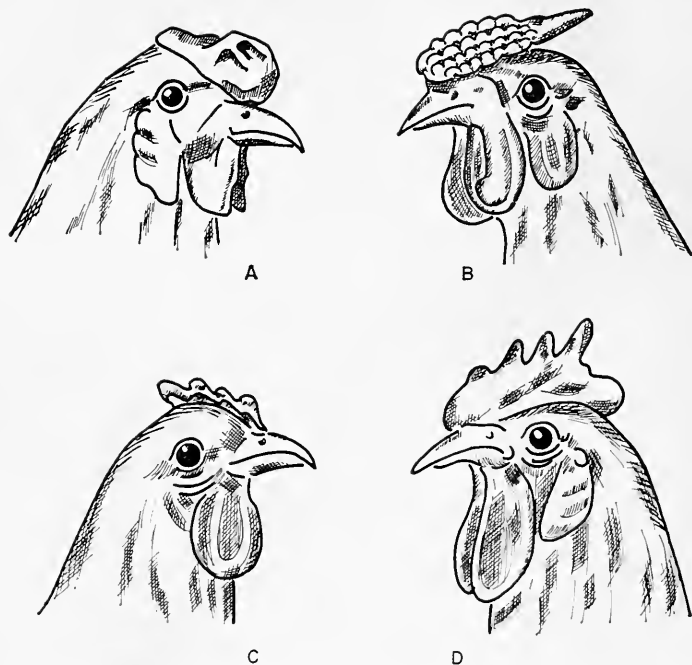


FIG. 9-2 Comb shapes in poultry. (A) Walnut: $R/- P/-$; (B) rose: $R/- p/p$; (C) pea: $r/r P/-$; (D) single: $r/r p/p$.

single. Single is recessive to both rose and pea combs, in other words, a double recessive (Fig. 9-2).

A diagram of the mode of inheritance of rose and pea combs is shown in Table 9-8.

DUPLICATE GENES

We have discussed several types of genic interactions where the cooperation of more than one gene was necessary to produce a given phenotypic effect. It is also known in genetics that a certain phenotypic effect can be produced by *either* one *or* another given gene. One of the best illustrations of this was the work of George H. Shull. He is better known for his pioneer studies in hybrid corn, to be discussed in Chapter 12.

Shull, working in his botanical laboratory at Princeton University, made an intensive genetic study of a lowly weed, *Capsella bursapastoris*, a member of the mustard family, commonly known as shepherd's purse. He found that the regular triangular-shaped seed capsule was dominant to a small ovate shaped one. When the two were crossed, the F_1 was triangular, showing dominance. The F_2 generation gave a ratio of 15:1, instead of the 3:1 expected if the shape were due to a single gene. Further analysis by Shull revealed that

Table 9-8. Genetics of Rose and Pea Comb in Poultry

		P	
		rose R/R	pea r/r
		p/p	P/P
		\times	
		F_1	
		R/r	P/p
		$=$	walnut
		F_2	
P/p Segregation	Segregation for R/r	$\frac{3}{4} R/-$	$\frac{1}{4} r/r$
	$\frac{3}{4} P/-$	$\frac{9}{16} R/- \quad P/-$ walnut	$\frac{3}{16} r/r \quad P/-$ pea
	$\frac{1}{4} p/p$	$\frac{3}{16} R/- \quad p/p$ rose	$\frac{1}{16} r/r \quad p/p$ single

either of two genes could produce a triangular shaped pod. To produce a small ovate seed pod, it was necessary that both genes be homozygous recessive. These can be diagramed as in Table 9-9.

It is obvious that the only class lacking at least one dominant allele of either T_1 or T_2 is the lower right-hand square (9) with the genotype $t_1/t_1 \quad t_2/t_2$. Seed pods will be ovate, while all the rest are triangular in shape.

For convenience in identifying genotype, the different squares have been numbered from one to nine. The breeding behavior of these genotypes on self-pollination may be safely predicted (Table 9-10).

TRIPPLICATE GENES FOR A SINGLE CHARACTER

In wheat, triplicate genes have been observed for color of the seed coat of the kernel. Red is dominant to white, and in some crosses the F_2 generation produces a segregation ratio of three red to one white. In this case, only a single gene difference is involved.

In other crosses, the F_2 generation may show a 15:1 segregation or even a 63:1 ratio. The case of the 15:1 ratio is similar to that of the shepherd's

Table 9-9. Genetics of Duplicate Genes for Pod Shape in Shepherd's Purse

P			
T_1/T_1	T_2/T_2	\times	t_1/t_1 t_2/t_2
triangular pod			ovate pod
F ₁			
T_1/t_1	T_2/t_2		
triangular			
F ₂			
Segregation for T_1/t_1	$\frac{1}{4} T_1/T_1$	$\frac{1}{2} T_1/t_1$	$\frac{1}{4} t_1/t_1$
T_2/t_2 Segregation			
$\frac{1}{4} T_2/T_2$	$\frac{1}{16}$ T_1/T_1 T_2/T_2 (1) triangular	$\frac{1}{8}$ T_1/t_1 T_2/T_2 (2) triangular	$\frac{1}{16}$ t_1/t_1 T_2/T_2 (3) triangular
$\frac{1}{2} T_2/t_2$	$\frac{1}{8}$ T_1/T_1 T_2/t_2 (4) triangular	$\frac{1}{4}$ T_1/t_1 T_2/t_2 (5) triangular	$\frac{1}{8}$ t_1/t_1 T_2/t_2 (6) triangular
$\frac{1}{4} t_2/t_2$	$\frac{1}{16}$ T_1/T_1 t_2/t_2 (7) triangular	$\frac{1}{8}$ T_1/t_1 t_2/t_2 (8) triangular	$\frac{1}{16}$ t_1/t_1 t_2/t_2 (9) ovate shape

Table 9-10. Breeding Behavior of Genotypes in F₂ Segregation

Square Number	Individuals	Genotype	Breeding Behavior
(1)	1	T_1/T_1 T_2/T_2	Will breed true for triangular pod
(2)	2	T_1/t_1 T_2/T_2	Will breed true for triangular pod
(3)	1	t_1/t_1 T_2/T_2	Will breed true for triangular pod
(4)	2	T_1/T_1 T_2/t_2	Will breed true for triangular pod
(5)	4	T_1/t_1 T_2/t_2	Will give 15:1 ratio
(6)	2	t_1/t_1 T_2/t_2	Will give 3:1 ratio
(7)	1	T_1/T_1 t_2/t_2	Will breed true for triangular pod
(8)	2	T_1/t_1 t_2/t_2	Will give 3:1 ratio
(9)	1	t_1/t_1 t_2/t_2	Will breed true for ovate pod

purse in which there are two, or duplicate, genes. The dominant allele of each is capable of producing the effect. The seed coat color in wheat was carefully analyzed by H. Nilsson-Ehle, a Swedish geneticist and plant breeder early in this century.

In the case of the 63:1 ratio, there were three genes segregating. The dominant allele of any one of these three genes is capable of producing color. Hence the only plants bearing white seeds are those recessive for all three genes for seed-coat color.

The parents and the F_1 may be represented as follows:

P						
w_1/w_1	w_2/w_2 (white)	w_3/w_3	×	W_1/W_1	W_2/W_2 (red)	W_3/W_3
F_1						
	W_1/w_1	W_2/w_2 (red)		W_3/w_3		

Such an F_1 would produce 63 red-seeded plants in the F_2 to one with white seeds. The red-seeded plants would not all have the same genotype and would give different breeding results in the F_3 . The different genotypes can be calculated by the gametic checkerboard, with eight different gametes being formed, a rather cumbersome method. Or, the bracket method may be used—taking each gene pair separately, since they are all segregating independently.

By now the student should be sufficiently experienced in writing genotypes, so that he can dispense with both checkerboard and brackets and write the different genotypes directly. He is encouraged to try this as an exercise.

There will be 27 classes of genotypes in an F_2 with three genes segregating as shown in Table 9-11. The 19 different classes of 37 individuals ($37/64$ of the

Table 9-11. Summary of 27 Classes of Genotypes for Three Heterozygous Genes

Phenotypes	Classes	Individuals
All red	19	37
Seg. 63:1	1	8
Seg. 15:1	3	12
Seg. 3:1	3	6
All white	1	1
Total	27	64

F_2 population) have at least one of the three genes for red in the dominant homozygous condition. Consequently they breed true. There is only one class of eight individuals having all three genes heterozygous, a requisite for a 63:1 ratio. Three classes of 12 individuals have two genes heterozygous and the

other gene pair homozygous recessive, giving a 15:1 ratio. Three classes have but one of the color genes heterozygous, while the other two are recessive, resulting in a 3:1 ratio. Only one of the 64 has all three genes homozygous recessive, producing all white kernels.

There are three gene pairs concerned with the production of seed-coat color. Therefore it is possible to have from six dominant alleles (W) to none, as represented in the two homozygous parents W_1/W_1 W_2/W_2 W_3/W_3 and w_1/w_1 w_2/w_2 w_3/w_3 . In the F_2 , individuals are obtained varying from six dominant alleles to none. The number in each class can be picked out of a chart showing the segregation. The number also can be found by expanding the binomial $(a + b)^6$. The coefficient of each term represents the individuals with the number of dominant alleles shown by the exponent of a . The exponent of b will be the number of recessive alleles. Thus $(a + b)^6 = 1 a^6b^0 + 6 a^5b^1 + 15 a^4b^2 + 20 a^3b^3 + 15 a^2b^4 + 6 a^1b^5 + 1 a^0b^6$. The number of plants with any number of dominant alleles is given in Table 9-12.

Table 9-12. Number of Dominant Alleles in F_2 with Three Genes Segregating

Dominant Alleles	Number in F_2
6	1
5	6
4	15
3	20
2	15
1	6
0	1
<hr/>	
Total	64

CONCLUDING REMARKS

A number of cases of genic interaction have been presented, including complementary genes in sweet peas and in corn, also an inhibitor gene in corn. Duplicate genes in shepherd's purse and triplicate genes in wheat were shown. Interactions of genes to produce different shapes of combs in poultry, and the interesting case of complementary genes to produce high cyanide content in white clover have given insight into the way genes act to produce their results.

The most plausible explanation of the way genes act is that specific genes control the production of certain enzymes that are necessary for different biological processes within the cell. The specific genes do not produce their effects alone, but do so in cooperation with a host of other "normal" ones in the biological organism. It is by a study of the specific genes, where different phenotypic differences can be observed, that insight has been gained regarding their mode of function.

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PROBLEMS

9-1. Define the following terms:

<i>A</i> gene (maize)	pea comb (poultry)
aleurone layer	pericarp
anthocyanin	<i>Pr</i> gene (maize)
<i>C</i> gene (maize)	precursor
<i>C</i> gene (sweet pea)	<i>R</i> gene (maize)
<i>Cl</i> gene (maize)	<i>R</i> gene (sweet pea)
color inhibitor	rose comb (poultry)
complementary genes	single comb (poultry)
cyanide production (white clover)	substrate
duplicate genes	triplicate genes
endosperm	walnut comb (poultry)
limited phenotypic expression	<i>Y</i> gene (maize)

9-2. Identify the following scientists, giving a major contribution of each, with an approximate date.

Bateson, William	Nilsson-Ehle, H.
East, E. M.	Punnett, R. C.
Emerson, R. A.	Shull, George H.
Hayes, H. K.	

9-3. Three different genotypes in *Drosophila* have the same phenotype, a white eye. The genotypes are as follows: (a) White eye in chromosome 1, sex-linked. Female is *w/w*, male is *w/Y*. (b) *bw/bw, st/st*, the double recessive for brown (chromosome 2) and scarlet (chromosome 3). (c) *bw/bw, v/v* (female) or *bw/bw, v/Y*, a combination of the recessive for brown with the sex-linked eye color, vermilion.

A. If you were given these three white stocks, could you by making a single cross with a brown (*bw/bw*) male distinguish the three different white types? Show how each would react with the tester stock. Why, do you suppose, was a brown male used as a tester?

B. Show what results would be expected in the F_2 of your cross for each type. Assume 2000 flies in the F_2 , 1000 males and 1000 females.

9-4. In corn three dominant genes are necessary for aleurone color. The genotype *A/— C/— R/—* is colored. Any homozygous recessive for one gene is colorless. What ratio of colored to colorless kernels will be obtained in the following genetic types?

- (a) $A/a \ C/c \ R/r$ selfed
 (b) $A/a \ C/c \ R/r \times a/a \ c/c \ r/r$
 (c) $A/a \ C/c \ R/R$ selfed
 (d) $A/a \ C/C \ r/r \times A/a \ c/c \ R/R$
 (e) $A/A \ C/C \ R/r$ selfed

- 9-5. A white strain of the genotype $a/a \ C/C \ R/R \ Pr/Pr$ was crossed with a white strain that was recessive for c/c instead of a/a , and dominant for the other genes for aleurone color. Give the genotypes and phenotypes of the parents and F_1 , and also show the phenotypic ratio in the F_2 . Show how you obtained this ratio.
- 9-6. The same white strain used in Problem 9-5 was crossed with a purple stock, but one which had a yellow endosperm below the aleurone layer. Give the genotypes and phenotypes of the parents and F_1 . Also show the phenotypic ratio expected in the F_2 and how you obtained it.
- 9-7. A strain of corn with red aleurone (pr/pr) and white endosperm (y/y) was crossed with a stock with yellow endosperm, (Y/Y), recessive for the a gene (a/a) and dominant for all the other aleurone genes. Give the genotypes and phenotypes of the parents and the F_1 , and show the phenotypic ratio in the F_2 .
- 9-8. A corn plant which had colored seeds is crossed with three tester plants with the following results.

With $a/a \ C/C \ R/R$ it produced 50 per cent colored seeds.

With $a/a \ C/C \ r/r$ it produced 25 per cent colored seeds.

With $A/A \ c/c \ R/R$ it produced 50 per cent colored seeds.

What is the plant's genotype? What ratio would it give if self-pollinated? What ratio would it give when testcrossed?

- 9-9. You are given five colorless stocks whose genotypes for aleurone color are unknown. When crossed with each of the three basic colorless stocks given below, the following results are obtained.

	$\times a/a \ C/C \ R/R$	$\times A/A \ c/c \ R/R$	$\times A/A \ C/C \ r/r$
Unknown 1	colored	colorless	colorless
Unknown 2	colorless	colorless	colorless
Unknown 3	colored	colorless	50% colored
Unknown 4	colorless	50% colored	colored
Unknown 5	colorless	50% colored	50% colored

Write the complete genotype (for the three basic color genes) of each of the unknowns.

- 9-10. In poultry colored feathers are due to two complementary genes which have been labeled C and O . A dominant allele of each is necessary for color. All other combinations are white. Also there is a color inhibitor in poultry similar to the CI gene in maize. Birds which are I/I are white, even in the presence of C and O . The White Leghorn has the genotype $C/C \ O/O \ I/I$. White Wyandottes are $c/c \ O/O \ i/i$, white, and White Silkies are $C/C \ o/o \ i/i$. Answer the following questions based on this information:
- (a) Give the phenotype of an F_1 , between a White Wyandotte and White Silkie. What would be the proportion of the different phenotypes in the F_2 ?
- (b) Give the phenotypes of the F_1 and F_2 of a cross between a White Leghorn and a White Wyandotte.
- (c) Give the phenotypes of the F_1 and F_2 of a cross between a White Silkie and a White Leghorn.

(d) Give the phenotypes expected if the F_1 from the previous question (c) were crossed to a White Wyandotte.

9-11. In poultry the different comb types have the following genotypes.

walnut	$R/-$	$P/-$
rose comb	$R/-$	p/p
pea comb	r/r	$P/-$
single comb	r/r	p/p

Tell what ratios of comb types would be expected in the following crosses.

- (a) $R/r P/p \times R/R P/P$
- (b) $R/r P/p \times R/R p/p$
- (c) $r/r p/p \times R/r P/P$
- (d) $R/r P/p \times r/r p/p$
- (e) $R/r P/p \times r/r P/P$

9-12. In wheat there are triplicate dominant genes for red seed coat color in the kernel. Any one of these genes will produce some red color. A white wheat must be recessive for all three genes. What will be the ratios of F_2 plants with red and with white kernels when the following F_1 plants are allowed to self-pollinate?

- (a) $W^1/w^1 W^2/w^2 W^3/w^3$
- (b) $W^1/W^1 W^2/w^2 W^3/w^3$
- (c) $w^1/w^1 W^2/w^2 W^3/w^3$
- (d) $w^1/w^1 W^2/w^2 W^3/W^3$
- (e) $w^1/w^1 w^2/w^2 W^3/W^3$

9-13. If seed were saved from an F_1 heterozygous for all three triplicate genes, how many genotypes would there be in the F_2 ? How many of these would breed true for red? For white?

The ABC's of Coat Color Inheritance in Mammals

IN CHAPTER 9 we discussed the interaction of two or more genes to produce a given phenotypic effect. Coat color in many of our pets, as well as of wild animals, is dependent upon the teamwork of several genes. Since the three basic genes have been designated *A*, *B*, and *C*, this chapter is labeled the ABC's of coat color inheritance. Actually there are also *D* and *E*, in addition to these three basic genes.

THE *A* SERIES

The *A* allele is basically one concerning the pattern of pigment, either of the individual hairs or of the distribution of different colored hairs throughout the coat. The designation *A* comes from the *agouti*, a rodent of the guinea pig family about the size of a rabbit, common in tropical America. It has the protective coloring found in wild rabbits, squirrels, rats, mice, and other animals. The pigment pattern in this case applies mostly to the individual hairs, which are black except for a band of yellow near, but not at, the tip of the hair. This "subapical" band of yellow on an otherwise black hair gives a gray-brown coloration. Such a coat is more protective than one fully black or completely white. Wild animals of the agouti color are of the genotype *A/A*.

Occasionally in nature the mutation from *A* to *a* occurs. Such animals when homozygous *a/a* lack the yellow band on the hair and are completely black. This mutation has occurred in rabbits, mice, and rats and has produced completely black animals. Also, presumably, it has occurred in the common gray squirrel. A few years ago, I observed a coal-black squirrel in one of the parks of Washington, D. C. There seems little doubt that it was of the genotype *a/a*.

A^+ , A , a' , and a in Horses. In horses the pattern conditioned by the A allele is not one concerning the individual hairs, but rather the distribution of the hairs over the body. For example, in the bay horse, which is of the genotype A/A or A/a , the body color is reddish brown with black hairs limited to the mane, tail, and the lower portions of the legs. In this case the A allele limits the area of the body where black may appear.

In the wild Prejvalski horse, sole surviving remnant of the wild ancestral stock from which the domestic animals arose, the color is a neutral gray, comparable to the agouti color of the rodents. The genotype is A^+/A^+ . The reddish body color of our present bay horses is conditioned by other modifying genes in conjunction with the A/A or A/a , to be discussed in this chapter.

The mutation of A to a produces a horse that is entirely black when homozygous a/a . The body hairs as well as the mane and tail are black, since they lack the A allele.

Odriozola (1951) has postulated an a' allele in horses to account for the dark brown, or black-brown horse. Horses with the genotype a'/a' or a'/a are neither bay A — nor black a/a . They have a body color very dark brown or nearly black with lighter areas around the muzzle, in the flanks, and on the ventral portion of the body. The order of dominance seems to be $A \rightarrow a' \rightarrow a$. This interpretation has been accepted and elaborated by Castle and Singleton (1960).

A^s , a'' , and a' in Dogs. In dogs the dominant allele of A is commonly known as A^s . It allows dark pigment to extend over the entire body, giving black animals such as the black Labrador Retriever and the black Cocker Spaniel (Little, 1957).

Two other alleles of the A locus are known. These presumably have arisen by mutation from the A^s allele. The a'' allele for sable or tan color is recessive to A^s . Black pigment is replaced with one of reddish brown color. An excellent example of the effect of this allele is the Collie breed. The a' allele for the black-and-tan pattern (tan points) is the lowest member of the multiple allelic series at the A locus. Dogs with this coat color pattern have tan muzzles, feet, and under surfaces of tail, and also tan spots above the eyes. This is definitely a pattern gene, causing tan color to appear in the regions mentioned, regardless of whether the predominant color is black, brown, or sable.

The order of dominance in the A series in dogs is $A^s \rightarrow a'' \rightarrow a'$ (Little, 1957). Little also notes the possibility of another allele at the A locus, e.g., a^w , denoting the "wild color" similar to agouti in rodents, but caused by a recessive allele a^w/a^w . It would fall in the series between A^s and a'' , or between a'' and a' . Further data are necessary to establish this allele.

THE B SERIES

The alleles B and b are concerned with the color of pigment produced. If the genotype is B/B , or B/b , the dark pigment produced is black.

In the presence of b/b the pigment is brown, sometimes called chocolate, which it resembles. In horses b/b animals are known as chestnuts or sorrels. In rodents b/b animals are called brown or chocolate; if the agouti factor $A/-$ is present, they are known as cinnamon. Cinnamon animals differ from wild or agouti in having the individual hairs pigmented with brown and the subapical band yellow. This is in contrast to agouti animals that have black hairs with the subapical yellow band.

Animals that are a/a in addition to b/b have a uniformly brownish coat, with no pattern in pigment distribution.

Some confusion has arisen regarding the "brown" terminology. In rodents the brown refers to the chocolate brown of the genotype b/b . When horse breeders speak of brown they mean the very dark seal brown, almost black, which clearly contains much black pigment. Consequently these horses are $B/-$, rather than b/b . Their genotype is $a^t/a^t B/-$, or $a^t/a B-$, as pointed out previously.

There is only one known recessive allele, b , for the B locus. It undoubtedly arose by mutation from B .

THE C SERIES

In addition to the A and B genes, an animal to be fully pigmented must also be C/C or C/c . Consequently "wild," agouti-colored mice, rats, and rabbits must be $A/- B/- C/-$ with at least one dominant allele for the A , B , and C loci. If any of these animals is c/c it is an albino and has no pigment. The eyes appear pink because the lack of pigment allows the erythrocytes in the blood vessels to show. The mutation from C to c has occurred in mice, rabbits, rats, and guinea pigs. No true albino has ever been reported in horses, and they are extremely rare in dogs and cats (Little, 1957). Albinos also occur in other animals, even in man. Mutations from C to c have undoubtedly occurred in such wild animals as deer and squirrels. It was pointed out in Chapter 1 that a whole colony of white squirrels was developed in Olney, Illinois, from a pair of white squirrels liberated in 1905. It is not certain whether these animals have pink eyes and are true albinos (c/c), but it seems likely. A true albino squirrel was shot a few years ago in the Blue Ridge Mountains near Berryville, Virginia. Also, a white squirrel, presumably albino, has been seen near Berryville—while yet another was photographed in a park in Richmond, Virginia. So the mutation from C to c appears quite common in wild as well as domesticated animals. A white deer was shot in the fall of 1959 near Charlottesville, Virginia; it was possibly an albino.

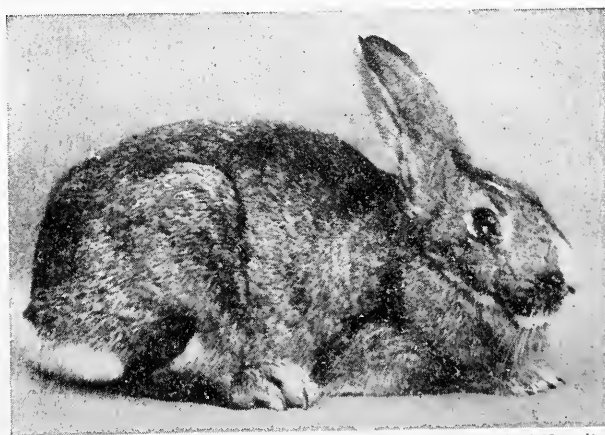
The albino allele in the homozygous condition (c/c) prevents the appearance of color, regardless of the genetic constitution of the other loci, A and B . Consequently albinos may be $A/- B/-$, $A/- b/b$, $a/a B/-$, or $a/a b/b$ in genetic constitution and will breed differently when the dominant allele C is added. The biochemistry of the albino mutant is now well understood. It

blocks the normal metabolic pathway in the production of melanin from tyrosine. This subject will be discussed in Chapter 19.

There are nine possible genotypes of albinos when only the genes A/a and B/b are considered along with c/c . A mating of albino $A/A B/B c/c$ by an agouti $A/A B/B C/C$ would produce an agouti F_1 with a segregation of $\frac{3}{4}$ agouti : $\frac{1}{4}$ albino in the F_2 . A mating of albino $a/a B/B c/c$ with agouti would produce an agouti F_1 . The F_2 would have $\frac{9}{16}$ agouti $A/— C/—$, $\frac{3}{16}$ black $a/a C/—$, and $\frac{4}{16}$ albino c/c , giving a 9:3:4 ratio. The albinos might have at least one A allele, but would be albino because of c/c . Since it is not possible to distinguish $A/— c/c$ and $a/a c/c$ individuals there are $\frac{4}{16}$ or $\frac{1}{4}$ albinos. Matings of some of the other types of albinos with wild type will be found in problems at the end of the chapter.

INTERMEDIATE ALLELES AT C LOCUS

In addition to the c allele, which precludes any pigment formation when homozygous, c/c , there are other alleles at this locus which alter the amount or kind of pigment formed. In rabbits, mice, and rats, the most common of these are Himalayan c^h and chinchilla c^{ch} . (The chinchilla allele also occurs in dogs.) The phenotypic effect of the chinchilla allele is to replace the yellow pigment with white. Thus an otherwise agouti animal, which is c^{ch}/c^{ch}



Courtesy of W. E. Castle; and Journal of Heredity

FIG. 10-1 Wild-type agouti rabbit $A/— B/— C/—$.

instead of $C/—$, has a silvery appearance because the yellow bands on the hairs have been replaced with white (no pigment). An example of this color, as well as agouti, Himalayan, and albino, is found in one of Castle's early publications (1924). These types are illustrated in Figs. 10-1 to 10-4. The allele c^{ch} is recessive to C .

The other allele in this series is Himalayan with the genotype c^h/c^h . Such

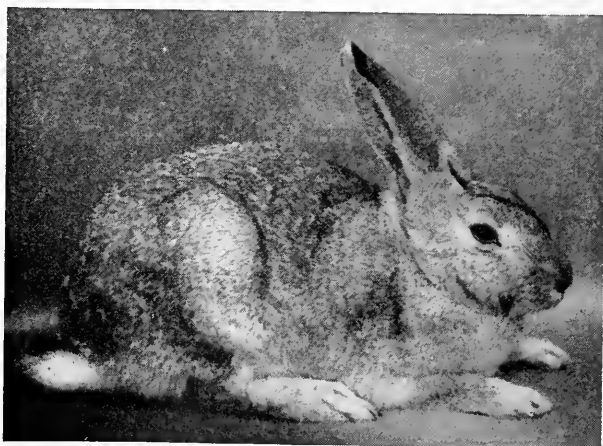


Courtesy of W. E. Castle; and Journal of Heredity

FIG. 10-2 Albino rabbit, c/c .

animals are mainly white, but with dark extremities—feet, ears, and the tip of the nose (Fig. 10-4). They are born white and the color develops as the animal matures. It has been found that this color is the result of an enzyme which is inactivated at body temperature. Consequently the young are born white and the color develops only in cooler portions of the body. Also, if the hair on any portion of the body is shaved off and the shaved portion kept cool with an ice pack the new hairs will be intensely pigmented.

A gene for Himalayan color has recently been reported in the mouse (Green, 1961). Like the condition in rabbits, the gene for Himalayan is an allele of the C locus and has the designation c^h . Homozygous Himalayan mice have a dark nose, ears, and tail, and a light but not white body (Fig. 10-5). The



Courtesy of W. E. Castle; and Journal of Heredity

FIG. 10-3 Chinchilla rabbit c^{ch}/c^{ch} .



Courtesy of W. E. Castle; and Journal of Heredity

FIG. 10-4 Himalayan rabbit c^h/c^h .

gene is recessive to the wild type (C), but forms intermediates with other alleles at this locus. F_1 animals, representing a cross between c^h and c^e (extreme dilution), had dark eyes at birth and a pale juvenile coat. At the first molt the ears, nose, and tail darkened, with the result that the extremities were darker than c^e/c^e but lighter than c^h/c^h , while the body was lighter than c^e/c^e but darker than c^h/c^h .

An F_1 , combining Himalayan with chinchilla (c^{ch}/c^h), that also was $a/a B/b$, had a light golden-brown color in contrast to the dull black or sepia color of homozygous c^{ch}/c^{ch} animals of comparable genotype. The noses and tails of the heterozygotes do not ever become as dark as in c^{ch}/c^{ch} . C/c^h animals are indistinguishable from C/C . In depth of color c^h is below c^{ch} in the series; it is above c^e in color of the extremities but below it in color of the body.

In crosses of the Himalayan rabbit with $A/A B/B C/C$ animals, the F_1 has a wild-type color and the F_2 segregates into three fourths wild and one fourth



Courtesy of Margaret Green; and Journal of Heredity

FIG. 10-5 Himalayan mouse.

Himalayan. The order of dominance of the various alleles at the *C* locus is $C \rightarrow c^{ch} \rightarrow c^h \rightarrow c$ (Table 10-1).

Table 10-1. Genotypes and Phenotypes of Different Alleles at the *C* Locus in Rabbits

Genotype			Phenotype
C/C			wild type
C/c^{ch} , c^{ch}/c^{ch}	C/c^h , c^h/c^h	C/c	wild type chinchilla
c^{ch}/c^h , c^h/c^h	c^{ch}/c		light gray Himalayan
c^h/c			Himalayan
c/c			albino

Another allele, c^e , one causing extreme dilution, occurs at least in dogs (Little, 1957) and in mice (Green and Dickie, 1959) and should be discussed here. In dogs the West Highland and White Terriers are of the genotype c^e/c^e . This gene acts to produce pups that are white at birth and remain nearly white as they mature, so that traces of light yellow pigment are hard to find and/or are definitely localized (Little, 1957). The positive identification of this allele is difficult. It can be confused with the action of the c^{ch} allele, which causes the color to be reduced to a light yellow, cream, or even white, as may be seen in Cocker Spaniels.

In horses, where no true albino, c/c , has been reported, there may be an intermediate mutant at the *C* locus. This allele c^{cr} for cream color is postulated by Odriozola (1951) to explain the coloration of palomino, buckskin, and dun horses. Most American geneticists follow the example of Castle in explaining these genetic types as due to a gene showing cumulative effects. This is labeled *D* for dilution. Thus d/d horses show no dilution. D/d animals have partial dilution to palomino, buckskin, or dun color. D/D horses show extreme dilution and are called cremello, if b/b , and perlino, if $B/-$.

The interpretation of Odriozola seems plausible and may be the correct one. Genetic tests to differentiate these two hypotheses are extremely difficult with an animal as slow breeding as the horse, and where no genes linked with the *c* locus are available for genetic tests. It seems logical that the gene *C* in horses might have mutated to an intermediate allele c^{cr} . No mutation from $C \rightarrow c$ is known in horses. In other animals, *C* has mutated to several alleles, c^{ch} , c^h , c^e , and *c*.

THE *D* SERIES

In this series the dilution gene is recessive so that animals must be d/d to show dilution of the coat color. It is not to be confused with the gene *D* for dilution in horses. In this case the gene for dilution is labeled *D*, indicating dominance. Actually there is no dominance, but rather a cumulative

effect. However, among horse geneticists, *D* indicates dilution. Only confusion would result if an attempt were made to change the terminology to conform to that for other animals in which the dilution gene *d* is recessive and is manifest only in homozygous individuals *d/d*.

In dogs there seems to be but one mutant type in addition to the *D*, the wild-type allele. Animals homozygous for the *d* allele have a uniformly diluted color, sometimes called the Maltese dilution. Good examples of *d/d* are found in dog breeds such as the Great Dane, Greyhound, Poodle, Chow, and particularly Weimaraner. Such animals have a silvery appearance, prominent in Weimaraners which are *b/b d/d* in genotype (Little, 1957).

In mice there is also a dilution allele *d*, as well as a dilute lethal *d'*. In both dogs and mice the *d* allele has no noticeable effect in the heterozygous condition *D/d*. Such animals are as fully pigmented as *D/D*.

In rabbits there is also a *d* mutant. Animals *d/d* in constitution have a diluted color and, with the genotype *A/A B/B d/d*, are distinctly blue in appearance. Such breeds as the American Blue, Imperial Blue, Blue Bevern, and Vienna Blue are of the genotype *d/d*.

THE *E* SERIES

The alleles at the *E* locus are concerned with the extension or restriction of black or brown pigment in the coat. When the dominant allele is present, there is no restriction of the areas where black or brown may appear. The distribution is then determined by which allele of the *A* series is present, since the gene for *A* concerns pattern. When the animal is homozygous recessive *e/e*, the black-brown pigment is replaced by red or yellow pigment. A good example of this in dogs is the Irish Setter, whose coat is a bright, rich red. The genotype is *A^s/A^s B/B e/e*. The suggestion has been made (Castle, 1954) that the mutant *e/e* might well be named for the Greek word *erythros*, meaning red. Possibly less confusion would result if this terminology were followed, rather than calling *e/e* a limitation of the extension of black or brown pigment.

In rabbits and guinea pigs the different combinations of *A/a*, *B/b*, and *E/e* alleles would produce the colors in Table 10-2 (after Castle, 1954).

Table 10-2. Genotypes and Phenotypes in Rabbits and Guinea Pigs

<i>A/—</i>	<i>B/—</i>	<i>E/—</i>	agouti	<i>A/—</i>	<i>b/b</i>	<i>E/—</i>	cinnamon
<i>A/—</i>	<i>B/—</i>	<i>e/e</i>	yellow	<i>A/—</i>	<i>b/b</i>	<i>e/e</i>	yellow, brown eyes
<i>a/a</i>	<i>B/—</i>	<i>E/—</i>	black	<i>a/a</i>	<i>b/b</i>	<i>E/—</i>	chocolate
<i>a/a</i>	<i>B/—</i>	<i>e/e</i>	sooty yellow	<i>a/a</i>	<i>b/b</i>	<i>e/e</i>	cream, brown eyes

In horses the different combinations of alleles of these three genes would appear as in Table 10-3.

Table 10-3. Genotypes and Phenotypes in Horses

$A^+/-$	$B/-$	$E/-$	Prejvalski, ancestral type, bay with zebra markings
$A/-$	$B/-$	$E/-$	dark or mealy bay
$A/-$	$B/-$	e/e	red bodied bay
a/a	$B/-$	$E/-$	recessive black
a/a	$B/-$	e/e	recessive black
$A/-$	b/b	$E/-$	chestnut
$A/-$	b/b	e/e	sorrel (light mane and tail)
a/a	b/b	$E/-$	liver chestnut
a/a	b/b	e/e	sorrel (uniform color)
a^t/a^t	$B/-$	$E/-$	seal brown
a^t/a	$B/-$	$E/-$	seal brown

DOMINANT BLACK (E^D) MUTANT IN MAMMALS

In several mammals a mutant form E^D increases the intensity of black pigment, even in the presence of A , the pattern gene. E/E^D animals are not as intensely pigmented as E^D/E^D , which are completely black. See Table 10-4.

Table 10-4. Phenotypic Expression of E^D Allele

a/a	$B/-$	E/E	recessive black
$A/-$	$B/-$	E/E	agouti
$A/-$	$B/-$	E/E^D	agouti—black showing only traces of agouti
$A/-$	$B/-$	E^D/E^D	dominant black, agouti pattern wholly concealed

According to Castle (1954), the dominant black is the commonest type of black found in Shetland ponies, which would account for the scarcity of bay animals. The phenotype of a bay animal $A/- B/- E/E$ would be changed to a dominant black if E^D alleles were substituted for the E allele.

SUMMARY OF A, B, C, D, AND E GENES

There is considerable similarity in the effects of these five genes in rodents, dogs, and horses. Apparently in many cases similar mutations have occurred in all of these species. Also, mutants for some of these genes have occurred in many other animals. The albino is such a mutation and occurs in man with a certain predictable frequency. Also, the mutation from E to e has apparently occurred in man, changing the hair color from the black-brown pigments to those in the red-yellow series. In man, red behaves as a recessive gene and might well be labeled e/e for erythros. It seems that this mutant is entirely similar to the e mutants in animals, for example, the red color of an Irish Setter. It is not surprising to find so many shades of red in human beings, when there exists the possibility of genic interaction with other alleles, similar to those studied extensively in mammals.

Table 10-5. Phenotypic Expression of Different Alleles in the Mouse, Rabbit, Dog, and Horse, Assuming Dominant Alleles of Other Genes

A SERIES				
Alleles	Mouse	Rabbit	Dog	Horse
A ⁺	—	—	—	wild-type Prejvalski; horse
A	agouti	agouti	A ^s dark throughout	bay
A ^Y	yellow (A ^Y /A ^Y lethal)	—	—	—
A ^w	white-bellied agouti	—	—	—
a ^y	—	—	sable, tan	—
a ^t	black and tan	black and tan	tan points	brown
a	non-agouti	non-agouti	—	black
a ^w	—	—	wild (agouti) color	—
a ^e	Extreme non-agouti	—	—	—
B SERIES				
Alleles				
B	black	black	black	bay, black extremities
b ^c	cordovan	—	—	—
b	brown	brown	brown	chestnut, sorrel
C SERIES				
Alleles				
C	colored	colored	colored	colored
c ^{ch}	chinchilla	chinchilla	chinchilla	—
c ^h	Himalayan	Himalayan	—	—
c ^e	extreme dilution	—	extreme dilution	—
c ^{cr}	—	—	—	cream dilution (Odriezola)
c	albino	albino	albino	—
D SERIES				
Alleles				
D	full color	full color	full color	—
D	—	—	—	dilute coat color
d	dilute	dilute	dilute	—
d ^l	dilute lethal	—	—	—
E SERIES				
Alleles				
E ^D /E ^D	—	dominant black no trace agouti	—	dominant black
E ^D /E	—	dominant black trace agouti	—	dominant black
E	black or brown full color	full color	full color	full color
e ^{br}	—	—	brindle	—
e	red-yellow	red-yellow	red-yellow	red-yellow

There are many differences in constitution of the five genes studied here. An attempt will be made to present the similarities in Table 10-5.

ADDITIONAL COMMENTS ON GENES FOR COAT COLOR

Combinations of different alleles of the different series produce some unusual phenotypes. The combination of c^{ch}/c^{ch} with e/e in rabbits is such an example. The c^{ch} allele limits rather markedly the yellow pigment in animals, while the gene e/e limits black, almost to the point of extinction. Consequently, in an animal with the genotype $c^{ch}/c^{ch} e/e$, the color is almost pure white. It is known as a "yellow chinchilla," but it is phenotypically white.

There is some inconsistency in the labeling of genes causing coat color dilution, as mentioned previously. In the D series there is a recessive dilution gene in several animals. However, the horse has a dilution gene, commonly called dominant and labeled D . It is a cumulative type with the heterozygote intermediate between the homozygous full color and the homozygous dilute. The designation D is in common use as the dilution gene producing palomino, dun, and buckskin horses. Apparently, this is a violation of priority rules in mammalian genetics, since d has long been used for recessive dilution in rodents. Perhaps more confusion would arise should an attempt be made to designate the dilution gene c^{cr} as suggested by Odriozolo (1951). His interpretation, though plausible, is inconclusive. There is in horses no demonstrated allele of a color gene with which we might make linkage tests of a dilution gene, whether we consider it to be an incomplete dominant or an incompletely recessive modifier of color (Castle and Singleton, 1961).

VARIABLE EXPRESSION OF D GENE: PENETRANCE AND EXPRESSIVITY

It has been pointed out that the phenotypic expression of the D gene in the heterozygous condition, D/d , is a palomino, or, in the presence of at least one B , a dun or buckskin. The expression of the D allele is remarkably constant. There are exceptions, however, in which the D allele fails to produce the usual phenotypic effect. Castle and King (1951) cited evidence that a mare described as black, when bred to a chestnut stallion, ($A/- b/b d/d$), produced a palomino foal. Evidently she was of the genotype $a/a B/b D/d$, although the D gene failed to produce any phenotypic expression. Such cases as this are said to be variations in *expressivity* of a given gene. Another way of describing this phenomenon is to say that the D allele in the exceptional case has low *penetrance* or no penetrance. If a gene produces a constant phenotypic effect, it is said to have 100% penetrance. Recessive genes could properly be said to have zero penetrance in a heterozygous individual. The penetrance may vary from zero to 100%.

The D allele is considered to have a very high penetrance, in that most animals possessing it show the characteristic diluted coat color. Because of the exception cited, the penetrance must be rated slightly less than 100%. A quantitative term for the amount of penetrance may be calculated by dividing the number of cases showing phenotypic expression by the total number of cases observed.

Another example showing lack of penetrance of the D alleles is shown in Fig. 10-6. The yearling in the center (Pegasus) is a cremello D/D in consti-

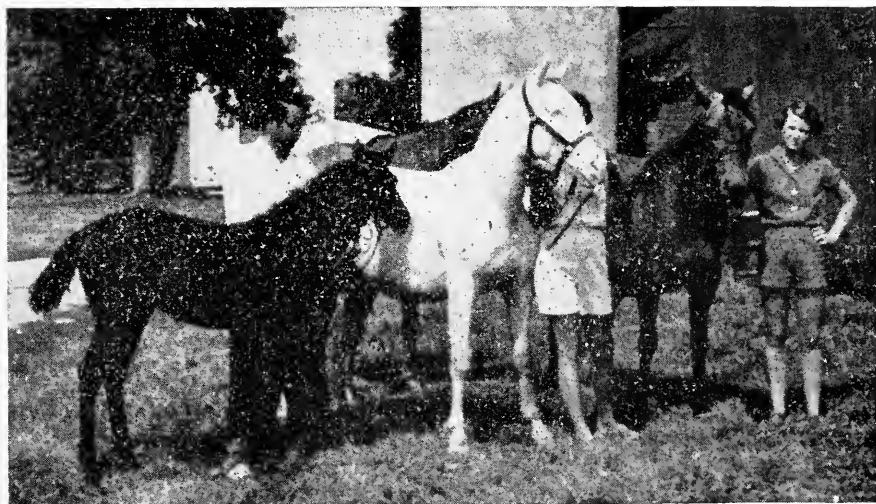


FIG. 10-6 Cremello $B/b D/D$ (center) with dun-colored dam (right) and brown full sister $a^t/- B/- d/d$ (left).

tution. His dam (right) is a dun whose genotype is $B/- D/d$. The sire of the cremello (Pegasus) is Poco Buck, a registered Quarter horse, whose color is listed as brown in the stud book. His color is similar to the brown filly also in Fig. 10-6, a full sister of Pegasus. Although Poco Buck is a brown phenotypically, he contributed a D allele to the cremello yearling and must be D/d . This is a clear case of no penetrance of the D allele in Poco Buck, which demonstrates that, while the expressivity of the D allele is constant in most cases, it did show variable expressivity in this instance.

INDEPENDENT GENES AFFECTING COAT COLOR

In addition to the five basic genes whose interactions produce the colors just discussed, there are a few genes that seem to produce their effects regardless of the genetic constitution of the five basic genes. In horses, the most important of these are the genes for gray (G), roan (R), and piebald spotting (S locus), and in dogs, "ticking" (T) and "merling" (M). The gene

T produces in white areas of the coat small flecks, or ticks of color, usually referred to as *ticking*. The gene *M* acts upon uniformly colored dogs to produce *merle* (dappled) animals. In horses there is also a dominant white (*W*), probably lethal when homozygous. The situation in dogs of *G/G* or *G/g* genotype is probably similar to horses, with pups not showing the influence of the *G* gene until several weeks old.

The gene for roan in horses (*R*) is dominant to solid color. *R/r* animals have white hairs interspersed with the normal colored hairs. It is not known whether *R/R* is lethal, but some think it may be. When *R* is superimposed upon a black background in horses, the color is known as blue roan. With bay it produces a red roan, and with chestnut or sorrel a strawberry roan.

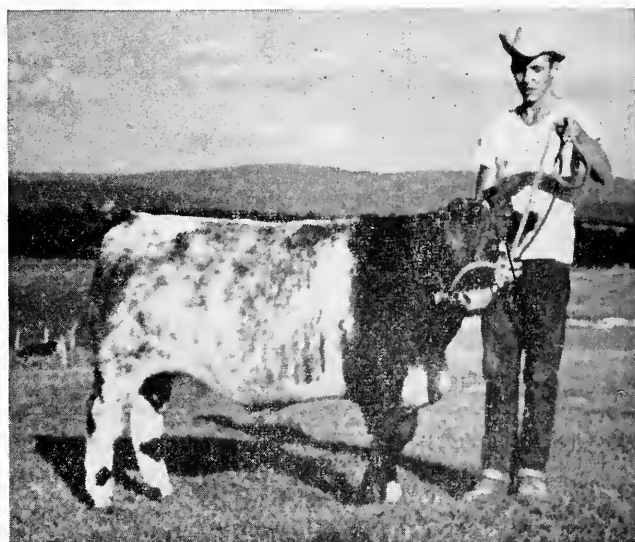


FIG. 10-7 Roan shorthorn steer. Note preponderance of red in fore part of animal.

In Shorthorn cattle a roan animal results when a red is crossed by a white. The F_1 is intermediate, with red and white hairs interspersed. Actually, the fore part of the animal has a greater preponderance of red than the rest of the body (Fig. 10-7). Neither red nor white is dominant. This might well be labeled with a system showing no dominance. A red would be C^R/C^R , a white one C^W/C^W , and the F_1 C^R/C^W , if *C* represents coat color.

EPISTATIC AND HYPOSTATIC GENES

The genes listed in the preceding paragraphs are sometimes called *epistatic* genes. This term was introduced by Bateson (1909) and serves a useful purpose if its use is limited to the original definition of Bateson. (See

Singleton, 1959, for a fuller discussion.) According to Bateson, the term epistatic (placed above) is applied to a gene that conceals or masks one or more other genes. The term *hypostatic* denotes "standing under" and is applied to the concealed, or masked, member of the series.

Bateson used the term epistatic to describe the masking of one gene by another not known to be allelic. As soon as two genes are shown to be alleles, the term dominance properly could be used. Actually the term *epistasis* (accent on the second syllable) has come to have a variety of meanings from "dominance of one gene over another that is non-allelic" to any type of genic interaction.

If the first of these two meanings is analyzed, we might ask: How can any gene be dominant to a nonallelic gene? The gene is dominant to its own recessive allele located at a particular site in a chromosome. A good example is the gene for gray color in horses. Animals that are G/g or G/G develop gray hairs which completely replace the original, whether black, brown, bay, or chestnut. The gene for gray G is prevalent in the Percheron breed of draft horses. Foals are born black and turn gray as the animal becomes several years old. This has given rise to the fallacious theory that all gray horses are black as foals and turn gray later. Actually a foal may be born black, bay, brown, chestnut, sorrel, or even palomino, and turn gray later if the gene for G is present. In Welsh ponies, where the gene for G is rather common, this change of color comes at a very early age. Hence it is necessary to record the color of a foal rather early to know the true phenotype, before it is covered or masked by the gene for gray (G).

The phenotypic expression of $G/—$ is a gray animal. Gray is not dominant to black, brown, bay, or chestnut, but to its own absence, or non-gray (g). The phenotypic expression of the g/g genotype is no gray hairs. The gene for G expresses itself regardless of any other gene present. In so doing, it masks or conceals the original color of the animal.

The second meaning of epistasis is that of genic interaction. However, the term "genic interaction" is much more descriptive than the other. The addition of the term epistasis to any known genic interaction does nothing to clarify or explain it. Hence in this context it seems entirely worthless. In coining the term, Bateson foresaw some confusion, but the trouble has exceeded anything he anticipated.

Part of the confusion results from failing to distinguish the inheritance of a given gene from its phenotypic expression. A good example is the c gene which produces albinos in a number of animals. The gene c is a recessive and shows good monogenic ratios in the F_2 of crosses with C . An animal that is c/c is albino, which precludes the formation of any pigment in the hair. In a sense, the hair color is masked, so that some authors prefer to call the interaction a recessive epistasis. If this is done, the gene for white eye in *Drosophila* should be labeled a recessive epistatic gene. To so label it does not explain it, but only creates confusion. Little misunderstanding would result if the original

definitions of Bateson were followed. In this text the terms epistatic and hypostatic are used according to his meaning.

THE GENE FOR PIEBALD SPOTTING

In dogs there are several alleles at the *S* locus (Little, 1957). These in order of dominance are:

- S* Solid color, completely pigmented body surface.
- sⁱ* Irish spotting, with few and definitely located white areas.
- s^p* Piebald spotting.
- s^{wp}* Extreme white piebald.

The three different types of spotting are illustrated in Little's book (1957). These are shown in Figs. 10-8, 10-9, and 10-10.

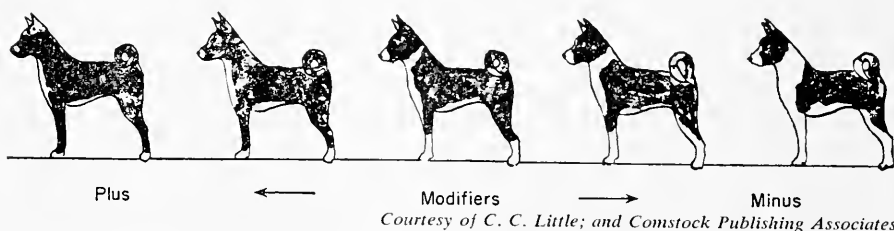
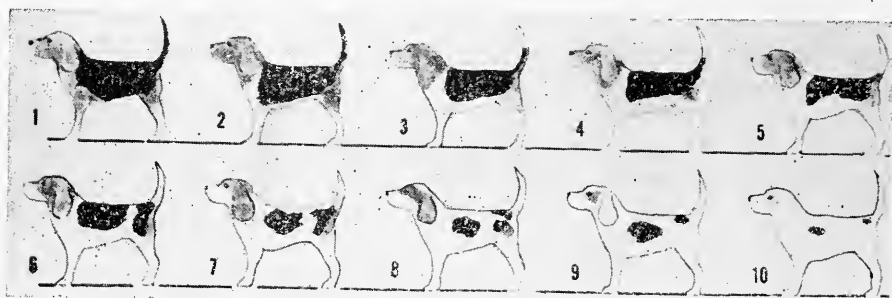


FIG. 10-8 Irish spotting (*sⁱ*) in Basenji breed of dogs, showing effect of modifying genes.

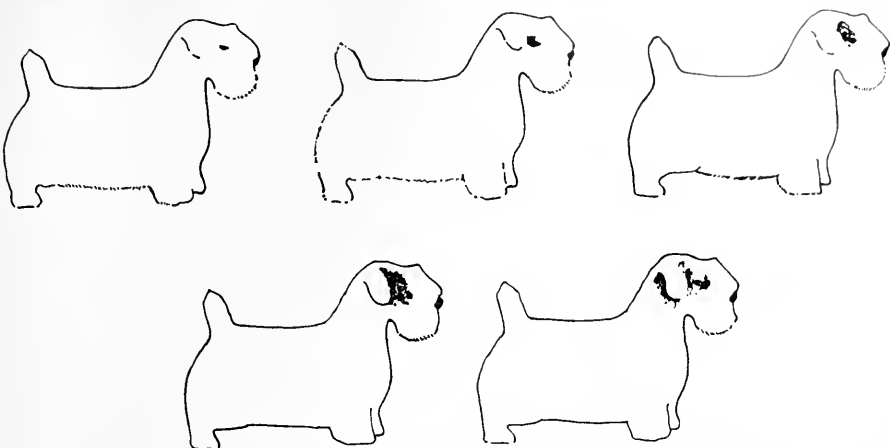
In horses there is a dominant gene producing a coat that has rather large white areas and colored areas scattered over the animal (Fig. 10-11). These are known to the horse breeders as *pinto* horses, from the Spanish word *pinto*, meaning painted.

There is also a recessive type of spotting in horses that produces white on the four feet and a blazed face characteristic of the Clydesdale draft horses



Courtesy of C. C. Little; and Comstock Publishing Associates

FIG. 10-9 Piebald spotting *s^p* in Beagle breed, showing wide range of piebald pattern, due to plus and minus modifiers.



Courtesy of C. C. Little; and Comstock Publishing Associates

FIG. 10-10 Extreme white spotting (s^{w}) in Sealyham dog breed. Animals with minus modifiers are pure white, while those with plus modifiers overlap with piebalds.

(Fig. 10-12). Also it results in various types of blazed face, varying from an almost completely white face to a star or a thin line down the face. This spotting is inherited as recessive to solid color.



FIG. 10-11 Dominant spotting in horses, commonly known as pinto.

DUTCH BELT AND OTHER TYPES OF SPOTTING IN CATTLE

In cattle, spotting occurs in which there is a band of white around the middle or near the fore part of the animal, known as a Dutch belt. It is inherited as a dominant with the gene designation S and is one of a multiple



Courtesy of Anheuser-Busch Inc., St. Louis

FIG. 10-12 Recessive spotting in Clydesdale horses, showing blazed face and white feet.

allelic series. The others are s^h , the Hereford-type spotting (Fig. 10-13); solid color, s^c ; and the Holstein-type spotting, s . The order of dominance is S , s^h , s^c , s . A dominant white mutant is known in England in the semi-wild park cattle.



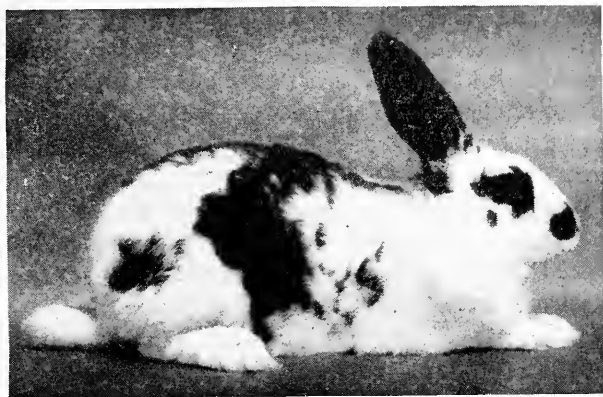
FIG. 10-13 Hereford-type dominant spotting.

DUTCH BELT AND OTHER SPOTTING IN RABBITS

In rabbits the Dutch belt type of spotting sometimes is manifest as a band around the animal, but at other times the white area is more extensive. This is inherited as a recessive (Castle, 1954). Another type is inherited as a dominant, and is known as English spotting (Fig. 10-14).

INHERITANCE OF COAT COLOR IN SWINE

In swine there is also a Dutch belt of white on a black background, which is characteristic of the Hampshire breed. This type is dominant



Courtesy of W. E. Castle; and Journal of Heredity

FIG. 10-14 English-type spotting in rabbits, a dominant mutant.

to a solid black or spotted such as occurs in the Poland China breed. Also, the spotted coloration found in the Poland China is dominant to solid color. Black is dominant to red.

The white color of the Yorkshire is epistatic to black. In a cross between a Hampshire and a Yorkshire, the F_1 is nearly all white. The Dutch Belt of the Hampshire is extended from the head to the tail, in some cases from the tip of the snout to a very small portion on the hind quarters. An F_1 sow of this cross was backcrossed to a Hampshire boar and produced a litter of approximately one Dutch belted to one solid white, confirming the epistatic character of the white color of the Yorkshire breed (Fig. 10-15).



FIG. 10-15 Four of a litter resulting from Hampshire-Yorkshire hybrid, backcrossed to Hampshire. Black pigs with white belt have color of Hampshire breed, and the white pig the color of Yorkshire. The backcross showed approximately 1:1 segregation. (All of litter is not pictured.)

GENOTYPES IN DOG BREEDS

Coat color in the dog has been studied genetically as thoroughly as in any of our domesticated animals. Also, the dog has a greater diversity

than any other animal. Since dogs are such popular pets, it seems pertinent to describe genetically a few of the well-known breeds. Our discussion is based upon the work of Little (1957), who has conducted extensive breeding experiments in the study of coat color in dogs. More than 4000 pups were produced and recorded at the Jackson Memorial Laboratory (Bar Harbor, Maine), in addition to records furnished to Little by many cooperators. Of the many breeds studied only a few will be presented below as genetic examples. Students are referred to Little for a more thorough treatment of inheritance of coat color in dogs. The breeds chosen as examples here are selected to illustrate the diversity of genotype rather than to represent the most popular breed. Actually, the public affections are fickle. The No. 1 breed of 1960 is not the same as in 1925, or even in 1959. By coincidence the breed described first in the following series, the Beagle, in 1959 was the most popular in America. In 1925, first choice was the Airedale, which was rated only 31st by 1959. Poodles were second in 1959, but first the next year.

GENE DISTRIBUTION IN DOG BREEDS

This list of gene distribution in ten different breeds, beginning with the Beagle, represents only a small portion of the total (86) studied by Little. (The system of tabulating the genes is his.) Explanation of the system

BEAGLE						
	A^s (rare)					S
						s^i
(common type)	a^t	B	C	D	E	s^p
						s^w
	a^y	b	c^{ch}	d	e	t

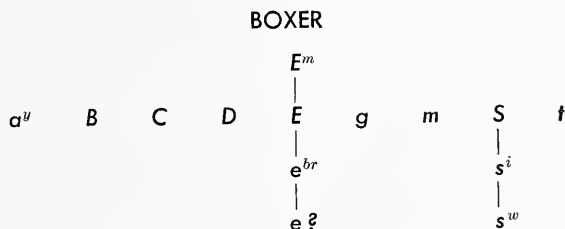
is as follows: The most commonly encountered alleles of the different genes (the type) are written on one line, with recessive alleles found on the line below. Two dominant alleles S and s^i over the s^p (piebald spotting) are placed above the line to indicate order of dominance.

The most common type of Beagle has a^t/a^t (dark saddle with tan points), while solid color (A^s) does occasionally occur. Also, sable (a^y) has been reported. Most Beagles are B/B (black), although there are liver-colored individuals b/b . The evidence for the c^{ch} (dilution) allele is that in some dogs the tan portion is pale, a flat buff rather than a rich tan. Most are C/C . Also, most dogs have full color, but a few dilute-blue individuals occur that must be d/d . The phenotype of most animals is $E/—$ (dark pigment), but in a few cases it is tan (e/e) with no dark pigment.

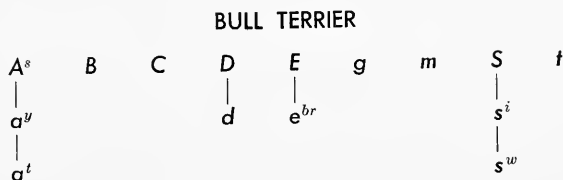
The most common type of spotting in Beagles is the piebald spotting, s^p , although Irish spotting (s^i) and extreme piebald (s^w) are found (Figs. 10-8,

10-9 and 10-10). The ticking character (T) seems to be present in many individuals, although others are t/t . Dogs that are $T/-$ have dark-colored flecks in an otherwise lighter area. The gene A^s is rare and actually may be a' with darkening and modifying genes.

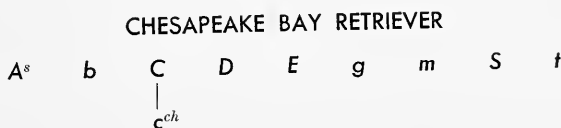
With these explanations in mind, the gene distribution of some other breeds can be studied.



The chief new allele encountered in the Boxer is E^m , which produces a black mask. It is dominant to E and has been placed above for this reason. The e^{br} is an allele that causes brindling when either e^{br}/e or e^{br}/e^{br} . The establishment of an e allele is in doubt, as indicated by a question mark. The g and m alleles indicate no graying or merling in the Boxer. The M gene has deleterious effects when homozygous. In Collies and in Shetland Sheepdogs, M/M individuals are commonly white, deaf, and/or blind, and often sterile as well. At the S locus, Boxers may be solid-colored, have the "Irish" type of spotting (s^i), or the extreme white piebald spotting, s^w . The t allele indicates no ticking.



The "white" bull terrier commonly seen is genetically s^w/s^w , which is really an extreme white form of piebald spotting.



The chocolate brown color of the Chesapeake Bay Retriever is due to the b/b gene. It will be noted that for most genes in this breed there is but one type. The one exception is the C locus where there are two alleles, C and c^{ch} . These two alleles are interesting from another standpoint, i.e., the heterozygous

C/c^{ch} is not deeply pigmented as the homozygote C/C . Little lists the following phenotypes for the different genotypes:

C/C	deep brown to medium
C/c^{ch}	medium brown
c^{ch}/c^{ch}	light brown (dead grass or straw)

Dominance of the C over the c^{ch} allele is lacking.

LABRADOR RETRIEVER

A^s	B	C	D	E	g	m	S	$T^?$
a^t	$b^?$	c^{ch}		e				t

The most common animals of the Labrador Retriever breed are entirely black.

GOLDEN RETRIEVER

A^s	B	C	f	e	g	m	S	t
		c^{ch}		E			$s^i^?$	

The color of the Golden Retriever is due to the interaction of the B/B and e/e . No b is present.

ENGLISH SETTER

A^s	B	C	D	E	g	m	s^p	T
a^t	b			e			$s^{w^?}$	t

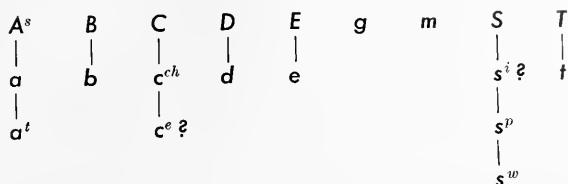
In the English Setter, the common spotting is due to s^p and animals may have the tan points due to a^t .

IRISH SETTER

				E				
A^s	B	C	D	e	g	m	S	t
a^y								
a^t								

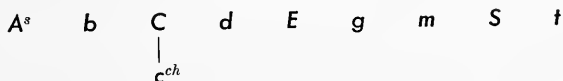
Note that the brilliant red color of the Irish Setter is brought about by e/e in combination with B/B , also A^s/A^s . All dogs are solid-colored (A^s) with no spotting.

COCKER SPANIEL



The chart of the Cocker Spaniel indicates why there is such a diversity of types, probably greater than in any other breed.

WEIMARANER



The genotype of the Weimaraner is in marked contrast to that of the Cocker Spaniel. In Weimaraners there is only one allele of all genes except at the C locus, where a c^{ch} allele is present in some individuals. Apparently those heterozygous for C/c^{ch} are lighter in color than C/C animals. The different genotypes with their phenotypes are as follows:

C/C	dark-colored
C/c^{ch}	medium-colored
c^{ch}/c^{ch}	light-colored

The Weimaraner has a rather unusual assortment of genes, in that it is homozygous for b/b , the brown gene, for d/d , the dilution gene, and also may be homozygous (or heterozygous) for c^{ch} , another dilution gene.

The fact that the C/c^{ch} genotype is intermediate to C/C and c^{ch}/c^{ch} shows that in some combinations there is no dominance, but a cumulative effect of the C allele. (This occurs also in the Chesapeake Bay Retriever, as noted previously.)

CONCLUDING REMARKS

The interaction of different genes to give a diversity of coat colors in mice, rabbits, dogs, and horses has been discussed. There are a great many similarities, indicating that similar mutations have arisen in all four species. These can be extended to other species as well. Certain differences exist between the species, but the similarities exceed the differences. The albino mutant (c), has occurred in man as well as in many other animals.

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PROBLEMS

10-1. Define or describe the following:

<i>A</i> locus (mammals)	erythros (Greek)
agouti	expressivity
<i>B</i> locus (mammals)	gray horses, genotype
bay horse	hypostatic gene
buckskin horse	liver chestnut horse
<i>C</i> locus (mammals)	merling in dogs
chestnut (horse)	Palomino horse
chocolate (mammals)	penetrance
cinnamon (mammals)	piebald spotting
Clydesdale horses	pinto horses
cremello horse	Prejvalski horse
<i>D</i> gene (horses)	roan cattle (Shorthorn breed)
<i>D</i> locus (other mammals)	roan horses
dominant black mammals	blue roan
dun horse	red roan
Dutch belted cows, pigs	strawberry roan
Dutch belted rabbits	seal brown horse
<i>E</i> locus (mammals)	sorrel horse
epistatic gene	ticking in dogs

10-2. Identify the following scientists, giving a major contribution of each, with the approximate date.

Bateson, William
Castle, W. E.

Little, C. C.
Odriozola, M.

10-3. A wild-type agouti female mouse was mated with a cinnamon male mouse, and produced a litter of five mice of different colors, two like the parents and also a black, chocolate, and albino. Write the genotypes of the parents and the F_1 , for three loci— A , B , and C .

10-4. The same agouti (wild) female was mated to an albino male and produced a litter of ten mice, of which six were albino and four agouti (like the mother). What is the most likely genotype of the albino?

10-5. The same agouti female mouse was later mated to an albino male and produced a litter of eight, half of them albinos, with one each of wild, cinnamon, chocolate, and black. What is the most likely genotype of the albino male parent?

10-6. There are nine possible genotypes of albinos when only the genes A/a and B/b are considered in conjunction with c/c , which produces albinism, regardless of the constitution for allele A and B . Write the nine different genotypes.

10-7. In a cross of an albino mouse with the genotype $a/a\ b/b\ c/c$, and a wild homozygous for all three alleles, what would be the phenotype of the F_1 ?

If 64 offspring were produced by mating the F_1 's and a perfect ratio obtained, how many of each phenotype would there be in the F_2 population of 64?

10-8. A dun mare when bred to a presumably brown stallion produced in two successive years a cremello stallion and a brown filly. Write the genotype of the dun mare and the "brown" stallion of the original mating.

10-9. Light gray rabbits can be either of two genotypes (Table 10-1). Mate each type by an albino male. Give the genotypes of the light gray animals and of the albino. Suppose each mating produced eight offspring and there was a random distribution of the different types. Give the phenotypes produced by each mating.

10-10. In rabbits there are four alleles at the C locus: C , c^{ch} , c^h and c . (The various genotypes and their phenotypes are given in Table 10-1.) A mating of a full-colored rabbit with a light gray one gave a litter of eight rabbits—four full color, two light gray, and two albino.

Write the genotypes of the parents and the F_1 , and give expected ratio.

10-11. A female mouse of the genotype $A^Y/a\ B/B\ C/C$ was mated to a male of the same genotype. What phenotypes would be observed in the progeny and in what proportion?

Quantitative Inheritance (Polygenic)

THE CHARACTERS studied previously are ones in which there is a distinct qualitative difference between genetic types. The characters are clear-cut and distinct, with no blending of gradation of one type into another. In most cases dominance is present, so that the homozygous A/A is phenotypically indistinguishable from the heterozygote A/a . In a few cases dominance is absent, and the heterozygote is intermediate between the two parents. This is true for some flower colors, where a cross between a red and a white produces a pink F_1 ; in crosses of red and white Shorthorn cattle, the F_1 is roan. Dominance is absent in the cross of special strains of black and white chickens, in the Blue Andalusian breed where the F_1 is a slate blue. It is also absent in the dilution gene that produces palomino color in horses, as was pointed out in Chapter 1. These characters are all conditioned by a single gene with no dominance of one allele over the other; yet the phenotypic expression is clear-cut and easily recognizable.

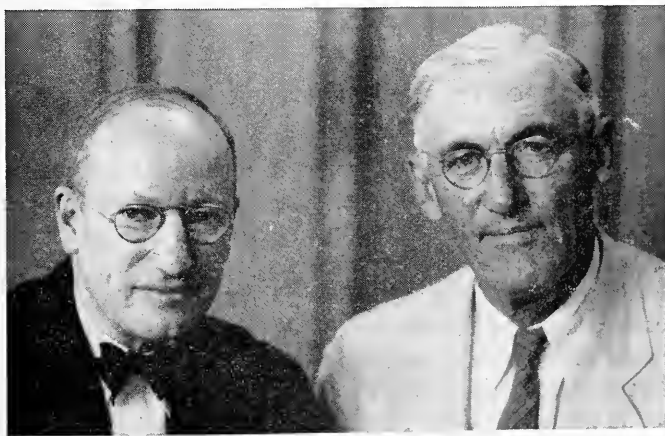
We have also considered teamwork among genes where the cooperation of two or more genes is necessary to produce the phenotypic effect. An excellent example of this is the complementary nature of two sets of genes in white sweet peas, which when crossed give a colored F_1 . A cross of $C/C\ r/r \times c/c\ R/R$ produces an F_1 that is fully colored, even though both parents are white. The same situation is found in aleurone color in maize, where the cooperation of four dominant alleles, A_1 , A_2 , C , and R , is necessary to produce color. In these cases the phenotypic expression, which is definite and clear-cut, is conditioned by the complementary action of two or more dominant genes.

Examples such as those mentioned, and many others, were studied during the first decade of the present century. The genetic ferment was great in those early years and, as is characteristic of such times, there were controversies. One of the big unsettled problems then was whether a Mendelian interpreta-

tion of inheritance applied to *all* characters, or only to those qualitative characters that showed a clear-cut segregation for distinct phenotypes in the segregating generation. What about a great number of characters (such as size of animal or plant, number of rows of corn, yielding capacity of crops, and many others), where the F_1 was more or less intermediate and a great segregation occurred in the F_2 without any division into separate discrete classes? Were such characters inherited on a Mendelian basis? These were serious questions before 1910.

In studying genetics we must not lose sight of the fact that other periods than ours had their exciting developments. Right now, 60 years after the re-discovery of Mendel's paper, some of the dramatic occurrences are the elucidation of the structure of the DNA molecule, the revelation of how mutations are brought about, and the story of the gene-enzyme relationship in understanding gene function. Earlier, around 1910, real progress was made in our understanding of quantitative inheritance.

The geneticist primarily responsible for the pioneer work and thought regarding the inheritance of quantitative characters was East (shown in Fig. 11-1 with Emerson) at the Bussey Institution of Harvard University. (This text is dedicated to him and to Castle, two of the author's teachers.)



Courtesy of Mrs. Trevor Teele

FIG. 11-1 E. M. East (left) and R. A. Emerson (right), pioneers in genetic studies of quantitative characters, at 5th International Genetics Congress, Ithaca, New York, 1932.

East was conducting his investigations at the same time that Nilsson-Ehle was developing the multi-gene hypothesis in cereals (Chapter 9). East found two genes for the yellow endosperm in maize. These he labeled Y_1 and Y_2 . A plant heterozygous for both of these genes, $Y_1/y_1 Y_2/y_2$, produced fifteen yellow to one white, similar to results found by Nilsson-Ehle. East did not conceive of these multi-genic segregations as limited to a few cases of seed

color in corn or cereals, but conceived of the idea that there may be a large number of genes influencing size when animals or plants of different sizes are crossed and that the increased size is due to the cumulative effect of many genes. This was an entirely new concept in genetics. In other words, he put the inheritance of quantitative characters on a Mendelian basis, even though an individual character might be influenced by several or many genes.

A conclusion of East's 1910 paper seems modern in its conception:

I may say in conclusion that the effect of the truth of this hypothesis would be to add another link to the increasing chain of evidence that the word mutation may properly be applied to any inherited variation, however small; and the word fluctuation should be restricted to those variations due to immediate environment which do not affect the germ cells, and which—it has been shown—are not inherited. In addition it gives a rational basis for the origin of new characters, which has hitherto been somewhat of a Mendelian stumbling-block, and also gives the term unit-character less of an irrevocably fixed entity conception, which is more in accord with other biological beliefs.

East was undoubtedly influenced in his thinking by the work of C. B. Davenport and G. C. Davenport at the Carnegie Institution in Cold Spring Harbor, New York. Davenport and Davenport (1910) and C. B. Davenport (1913) analyzed the skin color inheritance in the progeny of Negro-white matings. Their results could be satisfactorily explained by assuming that two genes affected color, with cumulative action.

DAVENPORT'S INTERPRETATION OF SKIN COLOR INHERITANCE IN MAN

The inheritance of skin color in matings of Negroes and whites can be interpreted on the basis of a two-gene difference (Table 11-1).

The number of individuals possessing any given number of alleles for color can also be found by expanding the binomial $(a + b)^4 = 1 a^4b^0 + 4 a^3b^1 + 6 a^2b^2 + 4 a^1b^3 + 1 a^0b^4$. In this example, a represents alleles for color with the exponent representing the number of such alleles. The coefficient of each term represents the number of individuals.

This scheme outlined in Table 11-1 may be an oversimplification of the case, in that all alleles for color may not contribute the same toward pigment formation. However, the hypothesis agrees fairly well with data obtained from the progeny of matings of Negroes and whites. The fact that so many shades of brown exist within mixed Negro-white populations is a good argument in favor of at least two pairs of genes for skin color determination. For more thorough treatment of this subject, the student is referred to *Human Genetics* by Curt Stern (1960).

SIZE DIFFERENCES IN MAIZE

Skin color in man has definitely been established as being caused by more than one gene for the different characters. What about such charac-

Table 11-1. Inheritance of Skin Color in Negro-White Crosses

P			
white (<i>a/a</i>	<i>b/b</i>)	×	Negro (<i>A/A</i> <i>B/B</i>)
F ₁			
<i>A/a</i>	<i>B/b</i>	mulatto (medium)	
F ₂			
Segregation for <i>A/a</i>	$\frac{1}{4}$ <i>A/A</i>	$\frac{1}{2}$ <i>A/a</i>	$\frac{1}{4}$ <i>a/a</i>
<i>B/b</i> Segregation			
$\frac{1}{4}$ <i>B/B</i>	$\frac{1}{16}$ <i>A/A</i> <i>B/B</i> black	$\frac{1}{8}$ <i>A/a</i> <i>B/B</i> dark	$\frac{1}{16}$ <i>a/a</i> <i>B/B</i> medium
$\frac{1}{2}$ <i>B/b</i>	$\frac{1}{8}$ <i>A/A</i> <i>B/b</i> dark	$\frac{1}{4}$ <i>A/a</i> <i>B/b</i> medium	$\frac{1}{8}$ <i>a/a</i> <i>B/b</i> light
$\frac{1}{4}$ <i>b/b</i>	$\frac{1}{16}$ <i>A/A</i> <i>b/b</i> medium	$\frac{1}{8}$ <i>A/a</i> <i>b/b</i> light	$\frac{1}{16}$ <i>a/a</i> <i>b/b</i> white

SUMMARY OF TYPES IN TABLE 11-1

No. of Alleles for Color	No. of Individuals	Phenotype
4	1	black
3	4	dark
2	6	medium
1	4	light
0	1	white

ters as ear length in corn, or corolla length in flowers? According to East's hypothesis, the inheritance of such characters was satisfactorily explained by the cumulative action of dominant alleles of several or many genes. His theory gave for the first time a Mendelian interpretation for the inheritance of quantitative characters.

A good example of this inheritance was the cross between Tom Thumb popcorn and Black Mexican sweet corn. The parents, F_1 and F_2 , are shown in Figs. 11-2 and 11-3, respectively, and by histograms in Fig. 11-4.

The F_1 hybrid was intermediate between the two parents with the mean slightly nearer Black Mexican with the longer ears. This indicated either dominance of the longer or, more likely, some hybrid vigor (*heterosis*) which



From East and Hayes, 1911

FIG. 11-2 Ear lengths (in cm.) of ears of Tom Thumb popcorn (above) and Black Mexican sweet corn (below).

would tend to produce ears slightly longer than expected. In the F_2 a symmetrical distribution was obtained, with the most frequent class (the *mode*) almost exactly intermediate to the two parents, and similar to the F_1 . The variation was much greater in the F_2 , with the extremes of the two parents not observed in a population of 646. In the F_2 , however, *means* (averages) of the two parents were obtained. That these were obtained in a population of 646 individuals is rather good evidence that ear length in this cross was dependent upon a small number of genes, perhaps not more than four or five. With four

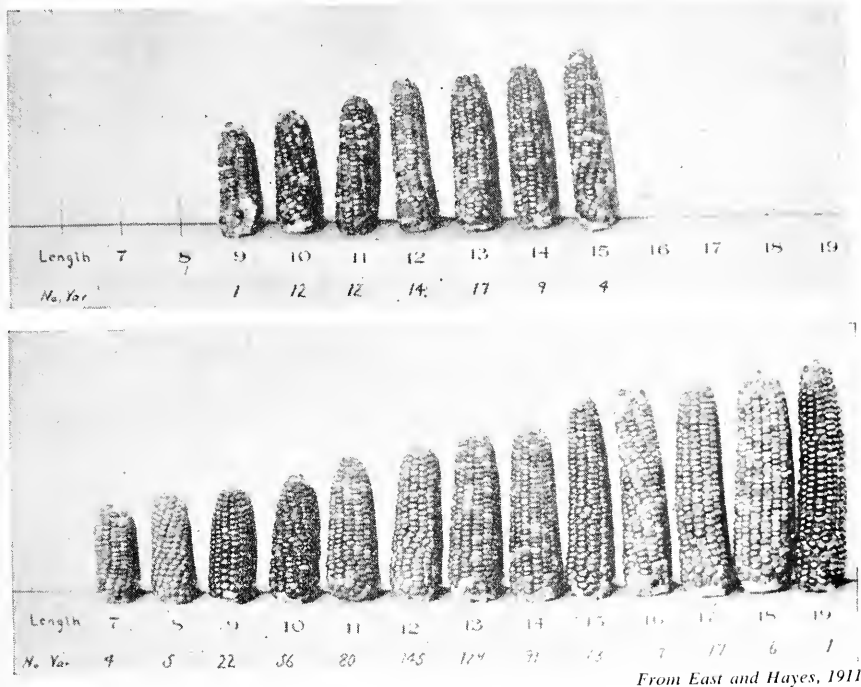


FIG. 11-3 Variation in ear length in F₁ hybrid (above) and F₂ generation (below) of cross of Tom Thumb popcorn and Black Mexican sweet corn.

genes segregating, a population of 256 individuals would be necessary to expect to recover one plant of each parental type. With five gene pairs segregating, a population of 1024 would be required.

COROLLA LENGTH IN NICOTIANA

Extreme differences in corolla length occur in *Nicotiana longiflora*. East studied the inheritance in corolla length between two varieties of this species, one with an average length of 40 mm., the other with 94 mm. length, a difference of more than 50 mm. The corolla length is a character that is influenced comparatively little by environmental conditions. Provided the plants have sufficient moisture, they will tolerate rather high differences in soil fertility without affecting materially the length of the flower. This makes them excellent material for studying the inheritance of quantitative characters, as East realized. This is the primary reason he chose corolla length in *Nicotiana* as his genetic material. The selection of material is extremely important if valid conclusions are to be obtained, as already pointed out by Mendel regarding his choice of peas.

The corolla length of the two parents and an average F₂ plant are shown

in Fig. 11-6 and also in a histogram (Fig. 11-7). The F_1 is intermediate between the parents with no flowers approaching the extremes of the two parents. Neither are the extremes of the parents recovered in an F_2 population of 389 individuals (Fig. 11-8), indicating more genes segregating for corolla

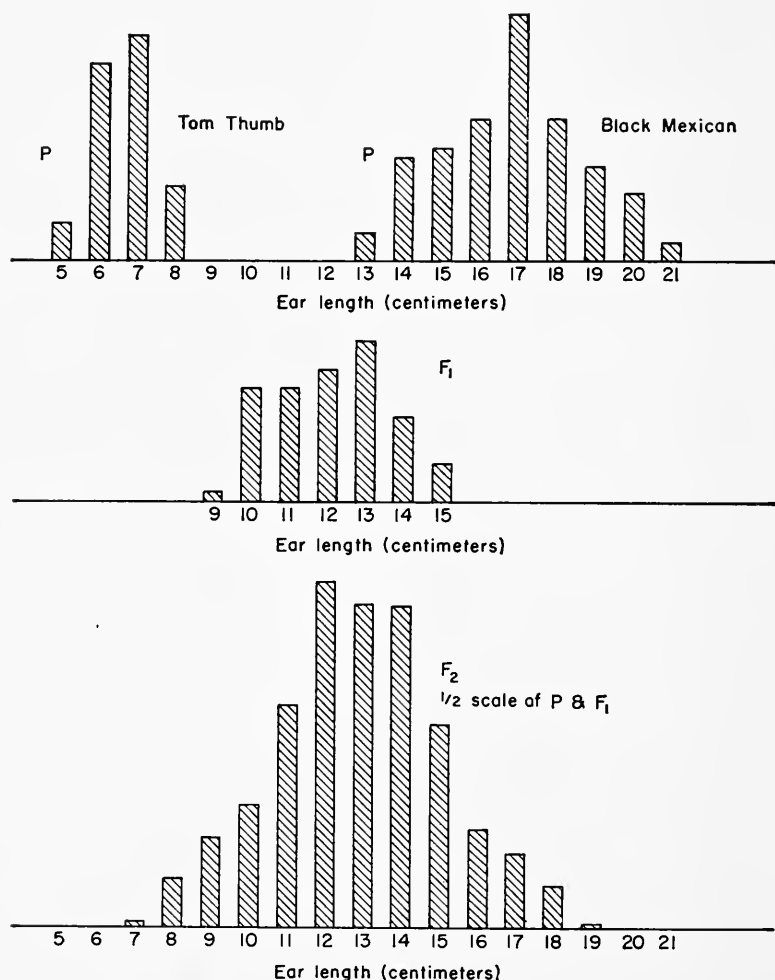


FIG. 11-4 Histogram showing ear length (in cm.) of Tom Thumb, Black Mexican, F_1 , and F_2 generations.

length than were observed for ear length in corn. Probably as many as six or eight gene pairs affecting length of corolla were segregating, possibly more. By selection it was possible in the F_5 generation to obtain individuals with corollas as diverse as the means of the two parents, which shows that the number of genes responsible was not infinite (Fig. 11-9).

NUMBER OF GENES SEGREGATING IN F_2

The difference in corolla length between the two varieties of *Nicotiana* was approximately 54 mm. Let us assign an arbitrary number of gene pairs segregating and see whether we can approximate the results obtained. If we assume six heterozygous genes, we would get from zero to twelve alleles for corolla length in the F_2 distribution. If we further assume that each allele adds an increment of $4\frac{1}{2}$ mm. to the corolla length, we should get a distribution similar to the one found in Table 11-2. These values are obtained by expanding the binomial $(a + b)^{12}$, since there will be from zero to twelve alleles for corolla length.

Thus we see the total is 4096, which is the minimum required for securing one specimen of each of the parental types. In other words, the chance is $\frac{2}{4096}$ that either parental type would be found in any F_2 population involving six pairs of genes (twelve alleles). It is possible that each allele would have a smaller effect on the phenotype. In this case more genes would need to be postulated to account for the results.



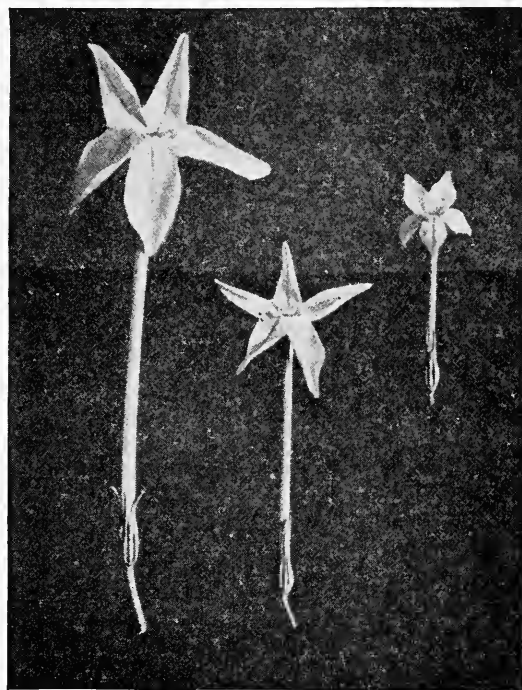
Courtesy of University of Minnesota

FIG. 11-5 H. K. Hayes, pioneer in the genetics of maize. He was for many years Chief of the Division of Agronomy and Plant Genetics, University of Minnesota.

Table 11-2. Expected Individuals in F_2 with 0-12 Alleles for Six Heterozygous Genes

No. of Alleles	No. of Individuals	Length of Corolla
12	1	94
11	12	$89\frac{1}{2}$
10	66	85
9	220	$80\frac{1}{2}$
8	495	76
7	792	$71\frac{1}{2}$
6	924	67
5	792	$62\frac{1}{2}$
4	495	58
3	220	$53\frac{1}{2}$
2	66	49
1	12	$44\frac{1}{2}$
0	1	40
Total		4096

Although the population of 389 individuals was not large enough to recover the parental types, it did produce some short flowers of 52 and 55 mm., and longer flowers of 82 and 85 mm. By looking at Table 11-2 we can see that such individuals should have three and ten alleles, respectively. In case of three alleles, they all might be different, and then would segregate in the next generation for three heterozygous gene pairs. In a population of 64 individuals, one would be expected with no alleles for increased corolla length. In other words, the original short parent would be recovered.



After East in Genetics

FIG. 11-6 Two varieties of *Nicotiana longiflora* showing an extreme difference in corolla length, with an average flower of F_2 generation in the center.

The plant with ten alleles for increased length must be homozygous for at least four of the alleles and possibly five. If the alleles for corolla length were distributed $4 + 1 + 1$ there would be two heterozygous pairs and one in sixteen of the F_3 should have flowers as long as the long parent.

Obviously this example is oversimplified. It is improbable that the alleles for increased flower length all have an effect of the same magnitude. However, the number of genes postulated could give the results obtained. This hypothetical explanation provides a method of showing why selection can be so effective in obtaining the desired types.

Selection really was remarkably effective, since East in the F_5 obtained plants whose flowers had the extreme corolla lengths of the parents. Regarding the efforts of the plant breeders to select diverse types, East pointed out, "Se-

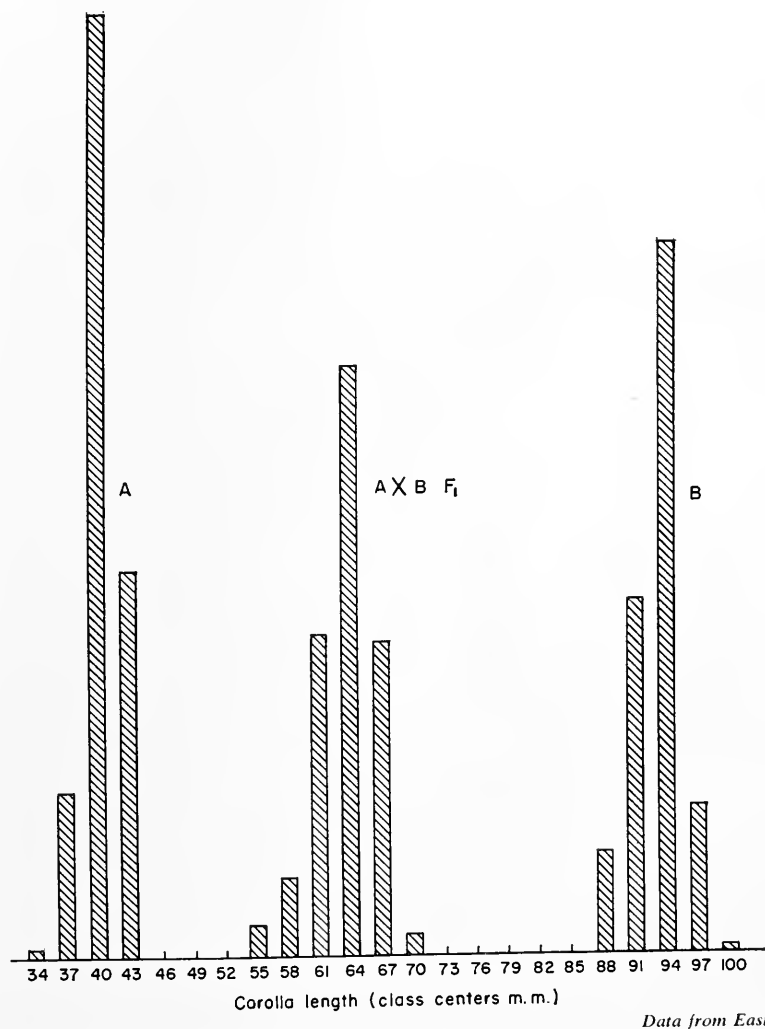


FIG. 11-7 Histogram of two varieties (A and B) of *N. longiflora*, with the F_1 hybrid between.

lection continued to several generations gives a high probability of success with comparatively little work." This is true only if the start is made with a mixed population consisting of many different genotypes, as he realized.

CONCLUDING REMARKS

During the first decade of genetics, 1900-1909, the inheritance of many qualitative characters was determined and the inheritance of such characters established as Mendelian. There was considerable doubt whether such quantitative characters as size differences were truly Mendelian.

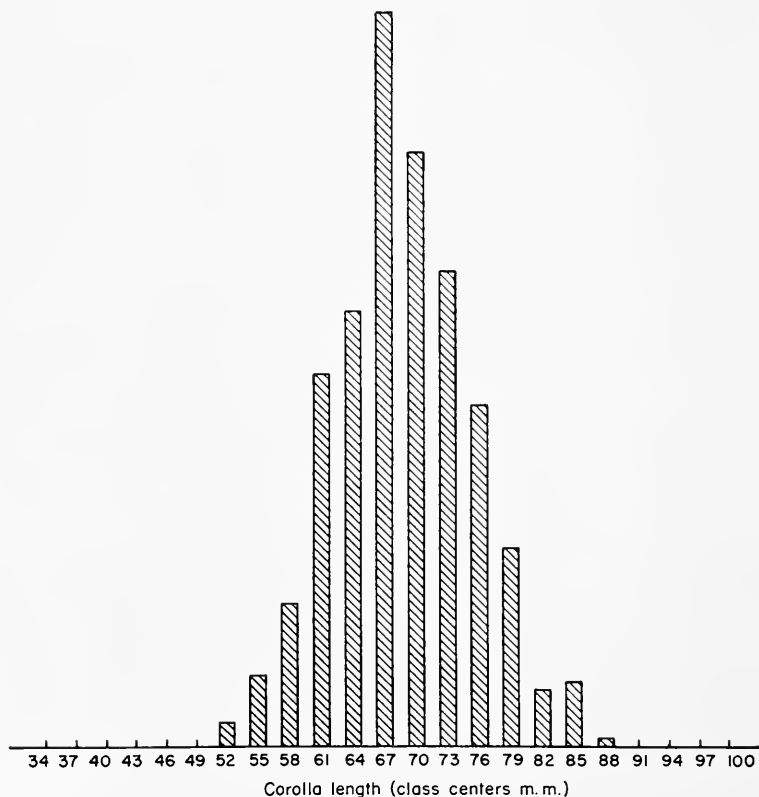


FIG. 11-8 Histogram of F_2 generation of cross between two varieties (A and B) of *N. longiflora* in Fig. 11-7.

At the end of the first decade, East proposed that quantitative characters were under genic control. He wrote that many genes affected the same character, such as size differences in animals or the inheritance of such characters as ear size in corn or the length of flowers in *Nicotiana*. His reasoning was based upon a careful analysis of the inheritance of quantitative characters in plants. Probably he was influenced by Davenport's genetic studies of skin color in Negro-white progeny. Davenport concluded that there were at least two genes for color involved. There was postulated a cumulative action of the

different alleles for color. With two genes for color it was possible to have from zero to four alleles present. He was able to recognize five distinct color classes.

The genetic analysis of skin pigment in man, ear length in corn, and flower length in *Nicotiana* provided the basis for East's hypothesis that all quantita-

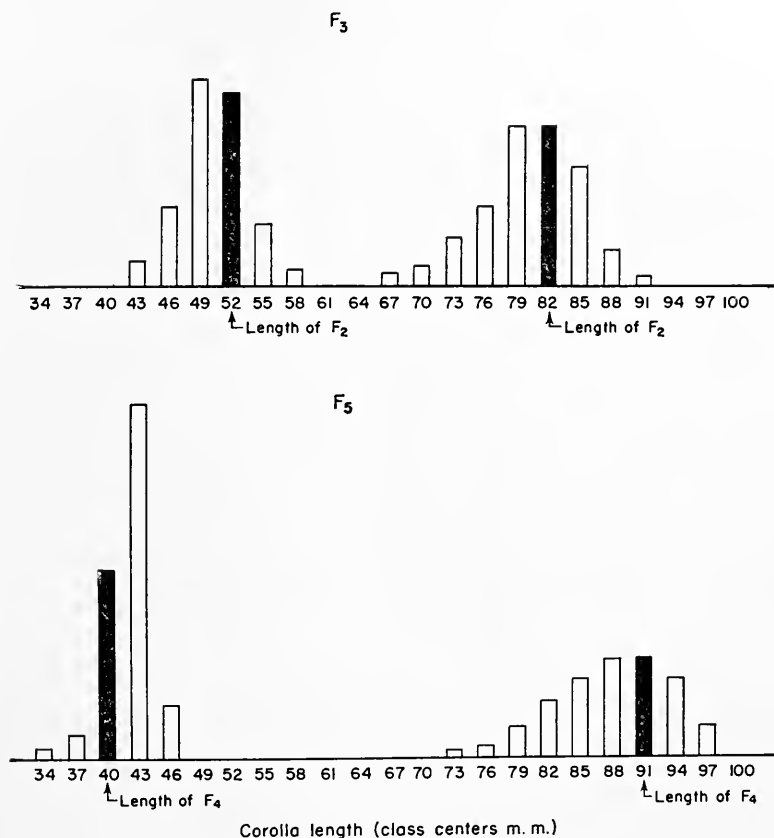


FIG. 11-9 (Above) F₃ generation of two different progenies from F₂ of hybrid between two varieties of *N. longiflora* (Fig. 11-8). The solid bars represent corolla length of F₂ plant selected to produce F₃. (Below) F₅ generation of two F₄ plants with different corolla lengths, indicated by solid bars.

tive characters are under genic control. Cumulative action of the different alleles provides the uniform gradations within an F₂ population. Thus the "blending" inheritance was shown to have a sound genetic basis. This was one of East's many contributions to the science of genetics.

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PROBLEMS

11-1. Define or describe the following:

DNA	genotype, white skin color
genotype, black skin color	mulatto
genotype, dark skin color	quantitative characters
genotype, light skin color	teamwork of genes
genotype, mulatto skin color	Y_1 and Y_2 in maize

11-2. Identify the following scientists, giving a major contribution of each, with an approximate date:

Davenport, C. B.	Hayes, H. K.
East, E. M.	Nillson-Ehle, H.
Emerson, R. A.	

11-3. Two homozygous genetics types of a hypothetical plant differed by 16 inches in height. Assume four pairs of genes responsible for the difference. The plant $a/a\ b/b\ c/c\ d/d$ is 10 inches tall, while the dominant $A/A\ B/B\ C/C\ D/D$ is 26 inches tall. Each capital letter adds two inches to the height. An F_1 , $A/a\ B/b\ C/c\ D/d$, would be 18 inches tall (10 inches plus 4×2 , or 8, = 18). In what proportion of the F_2 would the following classes for height occur? Express your answer as a fraction and not as a per cent. The different class heights in the F_2 should range from 10 inches to 26 inches, in even numbers only, since each dominant letter contributes two inches. Fill in the proper fraction of the following class heights.

Class	Height	Proportion
A	10 in.	
B	12 in.	
C	14 in.	
D	16 in.	
E	18 in.	
F	20 in.	
G	22 in.	
H	24 in.	
I	26 in.	

Classes A and I would breed true in later generations. What proportion of the F_2 population would breed true for height shown by class B? Class D? Class F? Class H?

11-4. In an F_2 population of a cross between Tom Thumb pop corn with an aver-

age of 7 cm. and Black Mexican with an average length of 17 cm., the averages of both parents were recovered once in a population of 400 (data of East and Hayes). If ear length promoting genes were of equal value and showed no dominance, how many genes would be necessary to explain the results? How many genes would be required to explain results if it were necessary to have a population of 1000 to recover the parental types?

- 11-5. Assuming the two gene hypothesis for the inheritance of skin color in Negro-white crosses to be correct, what is the darkest skin color that could result from mating of the following?

Matings	Darkest possible skin color
(a) dark \times dark	
(b) light \times dark	
(c) light \times light	
(d) light \times white	
(e) white \times white	

- 11-6. What is the lightest phenotype that could occur from the following matings?

Matings	Lightest possible segregate
(a) black \times black	
(b) black \times dark	
(c) black \times light	
(d) black \times white	
(e) dark \times dark	
(f) dark \times light	
(g) dark \times white	
(h) light \times light	
(i) light \times white	
(j) medium \times light	

- 11-7. In Table 11-2 of the text which classes of the F_2 individuals would breed true for height in succeeding generations?
- 11-8. How does Davenport's interpretation of skin color inheritance in Negro-white crosses compare with East's multigene interpretation of the inheritance of size differences in maize and *Nicotiana*?
- 11-9. Give briefly East's interpretation of the inheritance of quantitative characters.

Selection, Inbreeding and Crossing—Heterosis

HYBRID CORN is often cited as the outstanding contribution of science to agriculture. The production of field corn in the United States exceeded four billion bushels for the first time in 1959. This is enough to provide 22 bushels to every man, woman, and child in this country, or 100 pounds per month. It is produced on fewer acres than were used before hybrid corn, in fact, on only about three fourths of the acreage.

How did this phenomenal increase arise? What are the basic principles upon which the larger crop rests? The main reason that hybrid corn is superior to the open-pollinated varieties is that there is much better control over the heredity. The farmer can plant the same dependable hybrid genotype year after year, and in field after field, and the yield will be repeated. However, with open-pollinated types, varieties can fluctuate because there is little control over pollination. They are not predictable.

HYBRID CORN—CROSSES OF PURE LINES

All hybrid corn is a result of crossing comparatively pure lines. Since corn is normally open-pollinated, it is necessary to obtain pure lines by self-pollination before crosses are made. Once comparatively pure lines are obtained, they can be crossed in different combinations and the same hybrid can be produced and grown year after year with the same predictable results. This gives a much greater control over the heredity than is possible with open-pollinated varieties.

It should be pointed out that some of the plants in an open-pollinated corn field will be as robust and productive as the plants produced by crossing pure

lines. The difference between hybrids and open-pollinated varieties lies in the fact that a hybrid is much more uniform and will produce practically all of the plants of the same superior type, while it will be only an occasional plant in the open-pollinated field that will equal the hybrid. The results with hybrids can be duplicated year after year.

W. JOHANNSEN AND THE PURE LINE CONCEPT

Before discussing the production of pure lines in corn and their use in making productive hybrids, it seems desirable to present the classic studies of W. Johannsen on selection of pure lines in another plant, the common garden bean, *Phaseolus vulgaris*.

In the early 1900's Johannsen began his selection experiments with beans. He was interested in studying the value of selection for a quantitative character, seed weight. He wished to know how much the weight of seed was influenced by the genetic constitution and how much it was affected by the cultural conditions where the plant was grown.

As a result of his studies, Johannsen was able to distinguish the influence of the heredity from fluctuating variations that might affect seed weight. He coined two new terms "genotype" and "phenotype." The *genotype* is the genetic constitution of the individual, in this case a bean seed or a bean plant. *Phenotype* is a term for the physical appearance of the individual. These two terms, in common use by all geneticists, owe their origin to Johannsen's selection experiments with beans.

Johannsen's experiments were concerned with the value of selection. The character studied was seed weight. This is a quantitative character similar to flower length studied by East and described in Chapter 11. If we should weigh a large number of individual beans and plot a frequency distribution for the different weights, we would find a symmetrical distribution similar to that shown for flower length in *Nicotiana* (Fig. 11-8). A frequency curve for the beans used by Johannsen is shown in Fig. 12-1.

Actually these data represent all of the seeds grown from 19 individual pure lines of beans. The original selections were made in 1900. A year later, the 19 lines selected for further study had a total of 524 seeds. These seeds were weighed individually and accurate records kept in 1902, when 5494 seeds were produced. If the percentage of beans in each weight class is plotted against the average weight of each class, the frequency distribution shown in Fig. 12-1 is obtained. However, if the weights are plotted according to individual lines, a different picture unfolds. It is now apparent that the individual lines show considerable variation between them, and it is only by bulking all the data that a seemingly normal distribution is obtained. The smallest seeded line, No. 19, had a mean of 35 cg. per seed, while the heaviest seeded line, No. 1, averaged 64 cg. per seed, almost double that of the lightest. There was considerable variation in seed size in each of the lines, as is expected

for any quantitative character. All seeds produced by an approximately pure line follow a normal, symmetrical, frequency distribution; the total of all the individual lines make up the whole population.

Johannsen selected the lightest and heaviest seeds in each of the 19 different pure lines and planted them separately to see if the heaviest would produce heavier seeds than the progeny of the lightweight seeds. He found that within any of the 19 lines the weight of the mother seed had little or no influence on the weight of the progeny seed. This is shown graphically for four of the lines in Fig. 12-2. The remaining 15 lines gave similar results.

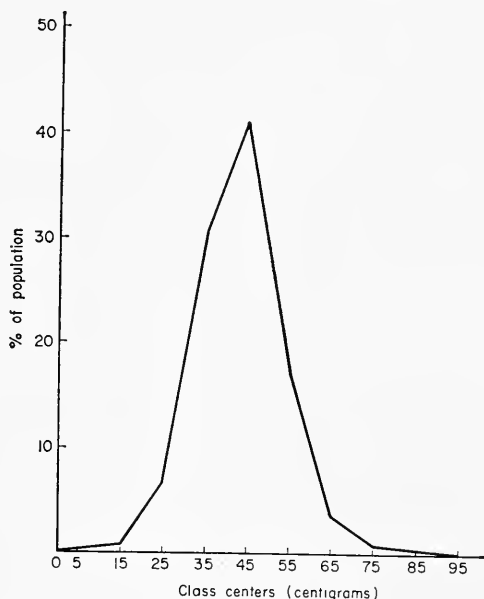


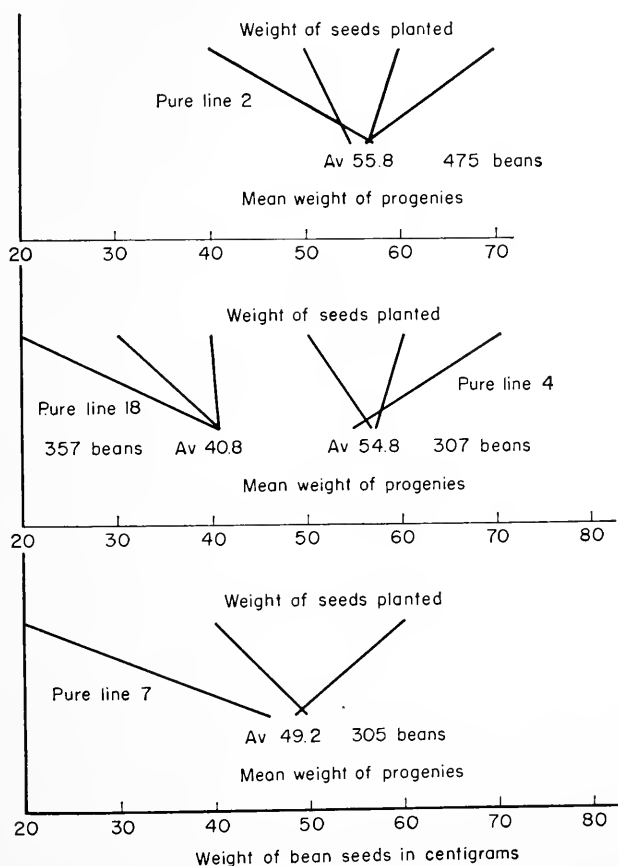
FIG. 12-1 Frequency curve of weights of 19 individual lines in W. Johannsen's bean experiments when lines were bulked. Actually, the large frequency graph is a composite of 19 pure lines, each with its own fluctuation.

One may observe in this illustration that seed with identical weights gave entirely different results. Take, for example, seed weighing 40 cg., shown in three of the four graphs (Fig. 12-2). In one case a 40 cg. seed (No. 2) produced a plant whose seed had a mean weight of 58 cg., while another 40 cg. bean (No. 18) produced a plant with a mean weight of 41 cg. In this case the bean seed planted represented the maximum weight for pure line 18. In this pure line the seed weighing but 20 cg., also gave a plant whose mean weight of seed was 41, the same as for the plant produced from a seed weighing double the amount.

Similar comparisons can be made for the four lines illustrated in Fig. 12-2

and for the 15 lines of Johanssen not shown graphically. Selection for seed size was of no value *within* a pure line.

Johanssen fully realized what was happening. The original beans represented a thoroughly mixed population of many pure lines. The size of the beans selected was determined in part by their heredity and in part by chance



Data from W. Johanssen

FIG. 12-2 Graphs showing ineffectiveness of selecting *within* pure lines. The heavy and light selected seed from a single progeny produced progenies with seed of similar weight.

fluctuation. By selecting seed of different sizes he was able to produce pure lines with greatly different average size of seed. Pure line 1 had an average of 64 cg. per seed, while the mean of line 19 was 35 cg. The explanation was obvious to Johanssen. His original selection was *among* different pure lines, while further selection was *within* lines. Since beans are self-pollinated, all of

the plants were essentially homozygous for all hereditary traits. At this point, Johanssen distinguished between the genetic constitution of the seeds, or genotype, and the physical appearance, or phenotype. It was fortunate for the science of genetics that Johanssen discerned so soon after genetic research began that these were not the same.

The genotype was not altered by the environment, or by chance fluctuation, whereas different environmental factors had a great influence on the size of the seed. Seeds of the same genotype could differ considerably in the phenotype—in this case the weight of each individual seed. Within a given genotype it was useless to try to influence the succeeding generation by selection for larger or smaller seeds.

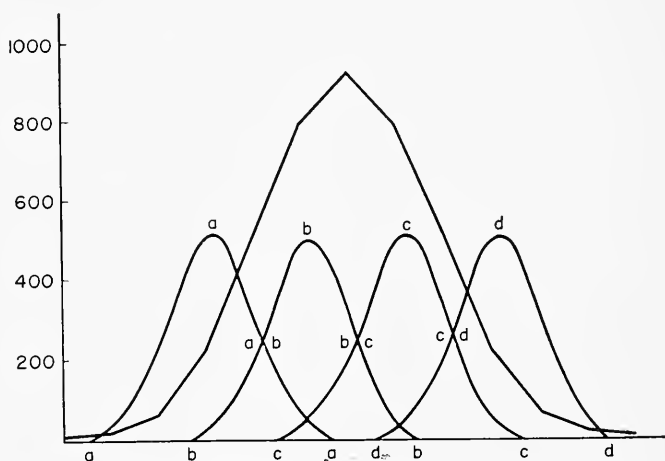


FIG. 12-3 Schematic representation showing how several pure lines, each with its frequency distribution, may be bulked to give one apparently symmetrical distribution. This is comparable to the data obtained by Johanssen.

The frequency distribution found by Johanssen (Fig. 12-2) actually was a composite of several symmetrical frequency distributions for the different pure lines. This is represented schematically in Fig. 12-3.

The genotype of hypothetical line *a* has a normal frequency distribution, as shown in *a-a*, while line *b* has a distribution of *b-b*. Actually there is a place where these two frequency distributions overlap. What about the beans in this area, the triangle bounded by the letters *b-ab-a*? Beans in this area could belong equally well to genotype *a* or genotype *b*. Phenotypically, they are nearly alike, similar in weight. The only way to determine the genotype of each would be to grow the seeds and see whether they would give a frequency distribution similar to *a-a*, or *b-b*.

The lower graphs in Fig. 12-3 show that there is some overlap even between genotypes *a*, *b*, and *c* in the small triangle *c-ac-a*. Seeds in this class are prac-

tically identical phenotypically, but could undoubtedly be separated into the three different genotypes *a*, *b*, and *c* by testing the progeny.

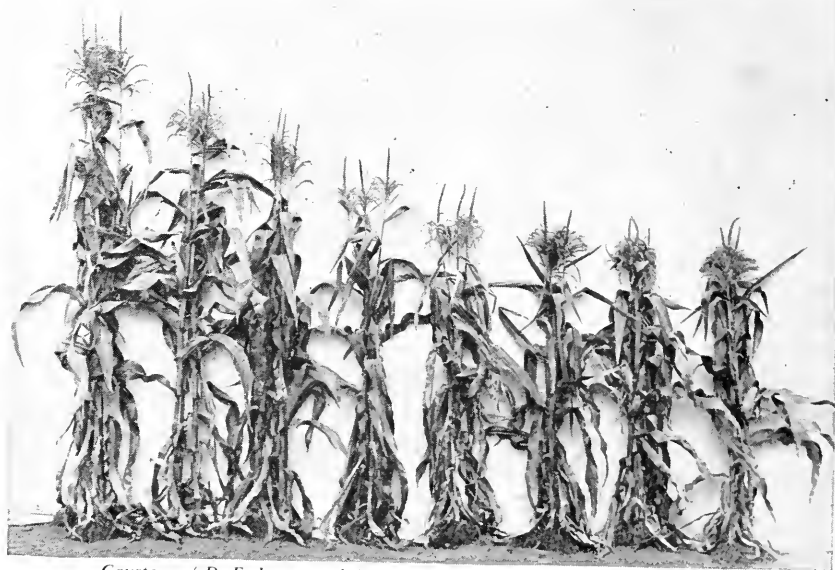
SIGNIFICANCE OF JOHANNSEN'S EXPERIMENT

Johannsen, like Mendel, selected his experimental material carefully, kept accurate records, and was able to devise a correct conceptual scheme to explain his results. He clearly differentiated hereditary variations from fluctuations that are not inherited. He showed that selection is valuable if there is genetic diversity in the material at the beginning of the selection. Once homozygous lines have been isolated, there is little value in selecting within a given line. Selection must be *among* lines rather than *within* them. The selection does not create the variation, but only acts on that present. His remarkable success in so short a time would have been impossible if he had not worked with a self-pollinated plant that had been reduced to homozygosity by countless generations of inbreeding.

INFLUENCE OF INBREEDING ON HOMOZYGOSITY

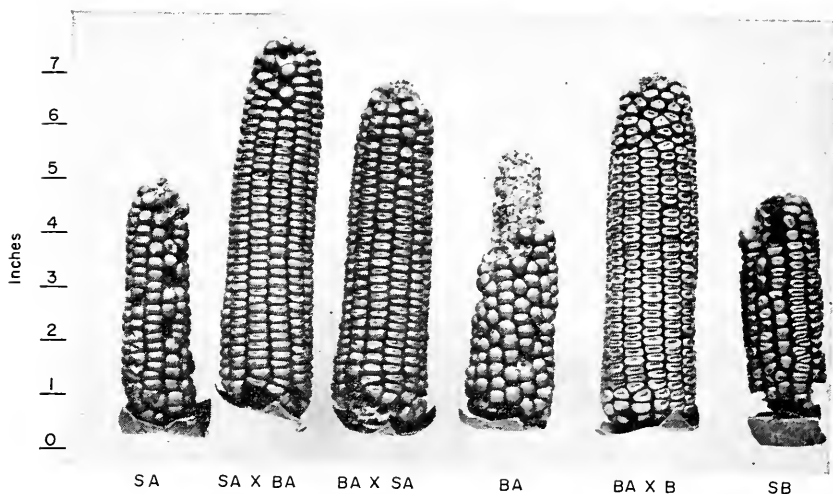
Self-fertilization is the closest kind of inbreeding possible and is accomplished in many plants. It can be induced artificially in many others. The corn plant is an excellent example of a plant in which artificial self-pollination has been extremely profitable. Pure lines in corn, similar to those in beans, have been isolated by controlled self-pollination. Some of this early experimental work was proceeding at the same time Johannsen was doing his classic work with pure lines in beans. Two investigators, East in Illinois and later at the Connecticut Agricultural Experiment Station, New Haven, and Shull at the Carnegie Institution for Experimental Evolution, Cold Spring Harbor, N. Y., began self-pollinating corn for different reasons, in 1904 and 1905, respectively. However, it was Shull who first realized what was being accomplished. This was the isolation of many pure lines, which he called *biotypes*. His paper "The Composition of a Field of Maize" is one of the classics of genetic literature. Another paper the following year, 1909, "A Pure Line Method of Corn Breeding" outlined the method later used for the production of hybrid seed.

Shull's experiments with corn were comparable to Johannsen's with beans. Johannsen had clearly demonstrated the isolation of pure lines in a self-pollinated crop. No artificial self-pollination was necessary—the beans did it themselves. In the case of corn, which is open-pollinated and consequently extremely heterozygous, there were no pure lines. By self-pollinating, commonly known as *selfing*, it was possible to produce pure lines, which Shull did. East also obtained pure lines in corn by selfing. Both Shull and East observed that the self-pollinated lines of corn decreased in vigor with successive generations of selfing. This is shown in Fig. 12-4, a photograph of a demonstration



Courtesy of D. F. Jones; and Connecticut Agricultural Experiment Station, New Haven

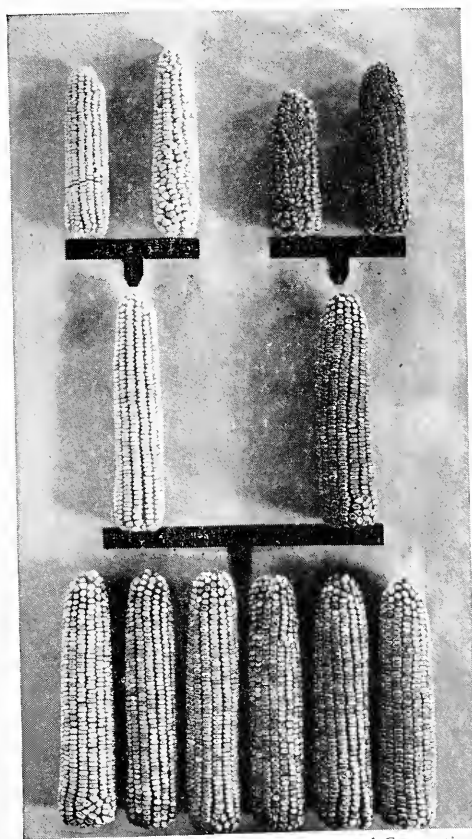
FIG. 12-4 Decrease in vigor upon inbreeding.



Courtesy of Connecticut Agricultural Experiment Station, New Haven

FIG. 12-5 Original inbred lines of George Shull and crosses between them. S A is Shull A; S B is Shull B; and B A is Shull's A line maintained a few generations by A. F. Blakeslee. Note vigor of cross of $A \times B$ and also line cross of two "different" A lines.

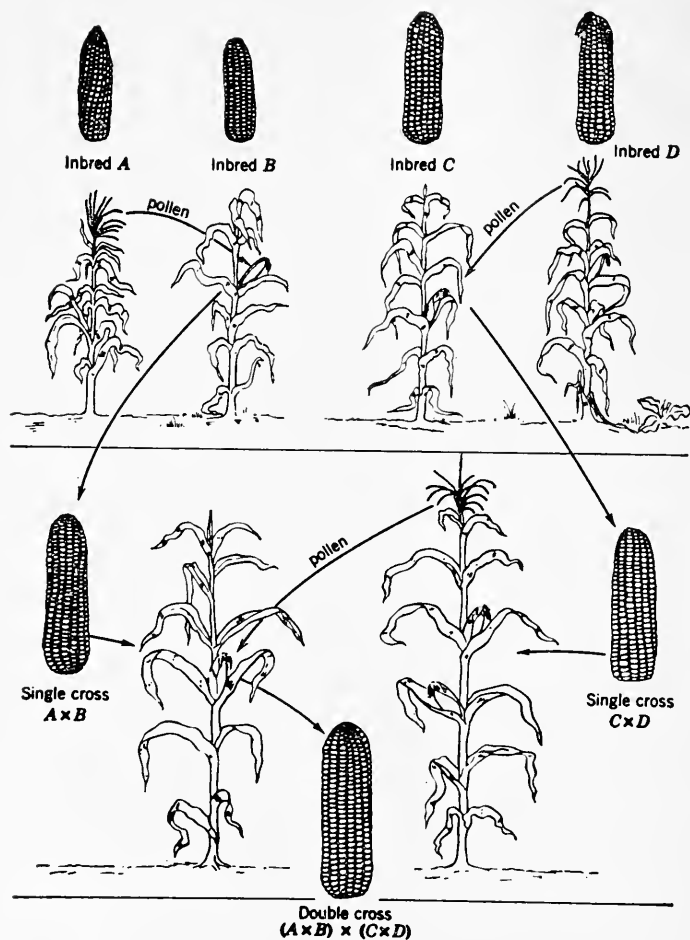
plot still grown every year by the Connecticut Agricultural Experiment Station. Had Shull and East stopped their researches with the production of pure lines, we should not have had hybrid corn as soon as we did. Instead, they crossed different lines and found an immediate increase in vigor. Shull's original inbreds and hybrids are shown in Fig. 12-5.



Courtesy of D. F. Jones; and Connecticut
Agricultural Experiment Station, New Haven

FIG. 12-6 Double crossed Burr Leaming, hybrid corn, the first double cross. Two white inbred ears were from Burr White, and the two yellow ones from inbred Leaming. Two F_1 are shown in center, with six ears of the double cross below.

As a result of a very few crosses, Shull proposed the pure line method in corn breeding in 1909. It had to be modified to obtain adequate seed yields. This modification, called the *double cross*, was made in 1917 by Donald F. Jones, who had received his graduate training with East at the Bussey Institution of Harvard University. The double cross consisted of making a hybrid between two different unrelated single crosses. Inbred $A \times B$ produces a single cross called AB . Also, $C \times D$ produces a single cross CD . The hybrid



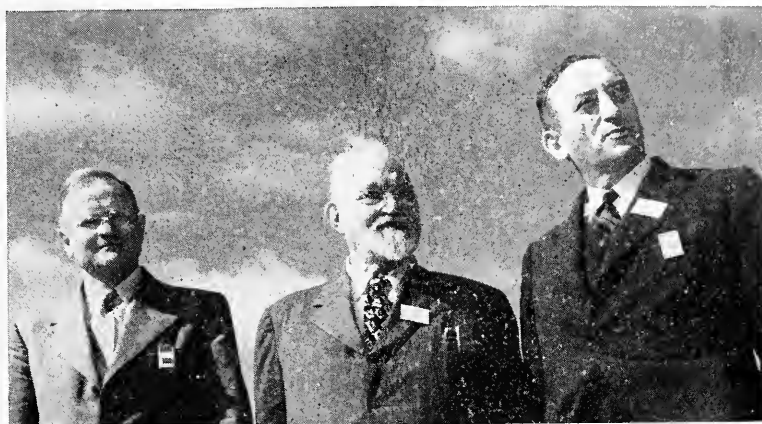
Courtesy of Th. Dobzhansky; and John Wiley and Sons

FIG. 12-7 Diagram of method for producing double cross.

between AB and CD gives a double cross, which combines in one hybrid the gene combinations from four separate inbred lines. This is illustrated in Fig. 12-6, with the original double cross, Burr-Leaming. The characters are diagrammed in Fig. 12-7. The inventor of the double cross, Jones, and of the single cross, Shull, are shown in Fig. 12-8, along with L. J. Stadler at a meeting of the Northeastern Corn Improvement Conference, Brookhaven National Laboratory, 1949.

SELF-FERTILIZATION AND DECREASED VIGOR

We have just observed that in the production of pure lines in corn there was a marked decrease in vigor. (No such decrease was noted in beans,



Courtesy of Brookhaven National Laboratory

FIG. 12-8 D. F. Jones, G. H. Shull, and L. J. Stadler, three investigators who made large contributions to our knowledge of the maize plant.

$$\begin{array}{c} P \\ A/A \times a/a \end{array}$$

Generations selfed	A/A	F_1 A/a	a/a	$A/A + aa$	
0		100%		0	0%
1	$1/4$	$2/4$	$1/4$	$1/2$	50%
2	$3/8$	$2/8$	$3/8$	$6/8$	75%
3	$7/16$	$2/16$	$7/16$	$14/16$	87.5%
4	$15/32$	$2/32$	$15/32$	$30/32$	93.75%
5	$31/64$	$2/64$	$31/64$	$62/64$	96.875%
6	$63/128$	$2/128$	$63/128$	$126/128$	98.45%
n	$\frac{(2^n)-1}{2^{n+1}}$	$\frac{2}{2^{n+1}}$	$\frac{(2^n)-1}{2^{n+1}}$	$\frac{2^{n+1}-2}{2^{n+1}}$	$\frac{100 \cdot 2^{n+1}-2}{2^{n+1}}$

FIG. 12-9 Increase in homozygosity upon self-pollination, with concomitant decrease in heterozygosity

since they were already pure lines at the beginning of the experiment.) Why should this decrease occur? The best explanation appears to be that a corn hybrid has much vigor because of many different dominant growth-promoting genes brought in by the two parents. As a hybrid is self-pollinated, which is the closest type of inbreeding, homozygosity increases and heterozygosity decreases. The homozygous inbreds, or pure lines, each contain fewer of the

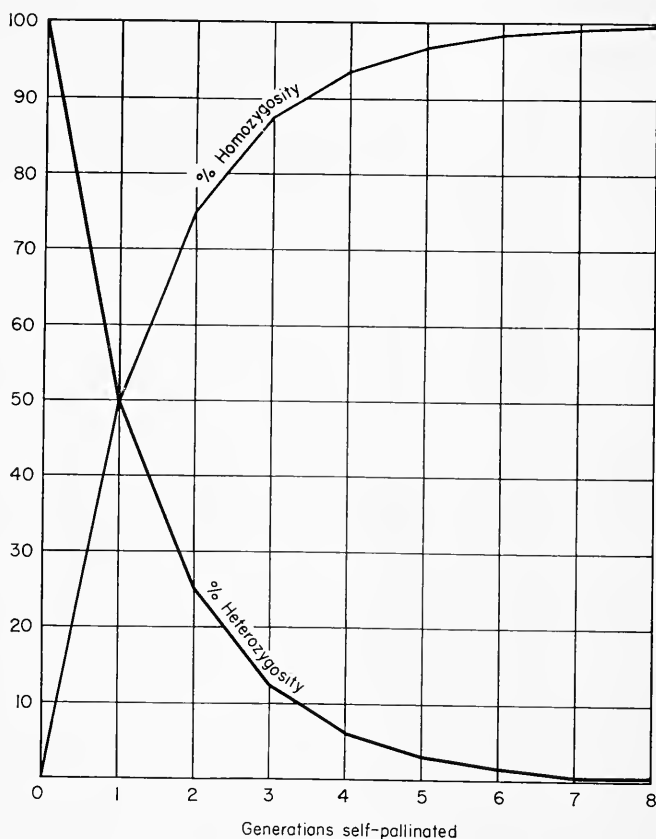


FIG. 12-10 Graph showing the effect of continued self-pollination upon homozygosity and heterozygosity.

dominant alleles than the F_1 hybrid. If dominance is present, i.e., one allele of A is equal to two, A/A , there is every reason for the F_1 to be vigorous, since it contains the maximum number of different dominant alleles. This is shown graphically in Figs. 12-9 and 12-10. After eight generations of self-pollination of a heterozygous individual, a single gene pair is changed from zero to more than 99 per cent homozygous. All genes taken separately would respond in the same manner. Hence it is not surprising to find such a rapid decrease in vigor upon inbreeding.

GENETIC EXPLANATION OF HYBRID VIGOR

Hybrid vigor has been explained by dominance of growth-promoting genes, so that a heterozygous individual A/a should be fully as vigorous as one containing both dominant alleles A/A . This was proposed by several workers, but its acceptance came largely following a paper by Jones, 1917, in which he proposed dominance of linked genes as the correct interpretation. The various interpretations have been reviewed by Singleton (1941).

Linkage was added to dominance by Jones to account for the observed fact that no inbred had ever been produced equal to the F_1 hybrid. Undoubtedly linkage is present. Corn has but 10 pairs of chromosomes, and any large number of genes for growth factors located in these ten chromosomes would necessarily show some linkage. It is conceivable that there could be at least 30 such genes, one at each end and one near the middle of each of the 10 chromosomes, showing very little effective linkage. If a corn plant were heterozygous for 30 growth-promoting genes, it would take an impossible corn field to grow enough plants to obtain a plant homozygous for all 30 dominant growth-promoting genes. The number of plants required to have an even chance of recovering such an individual would be 4^{30} . Such a corn field would require an area 2000 times the total land area of the earth.

Hence it is not surprising that no individual corn plant homozygous for all growth-promoting genes has been obtained. Even if one had a corn field large enough to have an opportunity for such a plant to be found, it would be virtually impossible to distinguish it from others that had an equal number of growth-promoting genes, some in the heterozygous condition. A great many genotypes would have similar phenotypes and consequently be indistinguishable.

The ready acceptance of Jones' theory, however, had a stimulating effect upon geneticists and corn breeders. Here was a logical explanation for the phenomenon of hybrid vigor, or *heterosis*. This was a term invented by Shull and used synonymously with hybrid vigor. Consequently, many more corn breeders began producing inbreds and hybrids, and the hybrid corn program advanced rapidly.

A second explanation, known as *overdominance*, has been advanced to explain hybrid vigor. This postulates that a heterozygous individual A/a is not only equal to A/A , but is slightly superior. A genetic interpretation of this phenomenon was proposed by East (1936). Possibly overdominance, as well as dominance, plays a part in causing hybrids to be superior to their inbred parents. Whatever the explanation, heterosis is playing an increasingly important part in increasing corn yields. In the Corn Belt states, hybrid corn is grown on 100 per cent of the corn acreage and the percentage is increasing rapidly in other sections of the country. It will not be too long before the corn production will be virtually 100 per cent hybrid, a remarkable accomplishment of the cooperation between basic and applied genetics.

PERMANENT HETEROSIS IN OENOTHERA

Heterosis, as originally used by Shull, is meant to be synonymous with the vigor usually found in hybrids. An unusual case of a perpetual hybrid condition in a seed-propagated plant is that of *Oenothera lamarckiana*, one of those studied by Hugo De Vries, the Dutch botanist, in working out his mutation theory. This plant is maintained in a hybrid condition upon self-pollination, by a balanced lethal system. The homozygous types are lethal and only the hybrid type of the *Oenothera lamarckiana* survives. This species is heterozygous for two complexes of genes, rather than for single genes. It was analyzed by O. Renner, who named the complexes *gaudens* and *velans*. The *gaudens* type has genes for green buds, broad leaves, and red flecks on the rosette leaves. The *velans* type contributes genes for red striped buds, narrow leaves, and no red flecks on the leaves. There are other differences, but these are the most noticeable.

(<i>gaudens-velans</i>)		
Eggs \ Sperm	gaudens	velans
	gaudens	velans
gaudens	gaudens-gaudens lethal	gaudens-velans
velans	gaudens-velans	velans-velans lethal

FIG. 12-11 Balanced lethal system in *Oenothera*, insuring cross pollination and the maintenance of a permanent hybrid with the usual hybrid vigor, or heterosis.

The species *O. lamarckiana* may be described as a *gaudens-velans* hybrid. These gene complexes apparently act as units and segregate as such. We might then expect from selfing *O. lamarckiana* to obtain one fourth *gaudens-gaudens*, one half *gaudens-velans* and one fourth *velans-velans*. However, only the *gaudens-velans* type is obtained; the other two homozygous ones apparently do not survive. This situation can be shown diagrammatically in the conventional checkerboard, or Punnett square (Fig. 12-11). Since the homozygous types are lethal, a permanent heterozygosis or heterosis is maintained. Apparently the *velans* and *gaudens* types carry different lethals, each having the dominant allele of the lethal of the other.

BALANCED LETHAL SYSTEM IN DROSOPHILA

A good example of a balanced lethal in *Drosophila*, whereby a hybrid condition is perpetually maintained, is the Curly Lobe Plum hybrid.

Curly (*Cy*) is a dominant wing character that causes the wings to be turned up. Lobe (*L*), also a dominant, causes an abnormal shape of the eyes. The gene Plum (*Pm*) produces a brownish eye color. All three of these genes are in the second chromosome. The chromosome carrying *Cy* and *L* contains two inversions that prevent crossing-over.

Flies that are heterozygous for these three genes breed true. The reason is that both homozygous types, *Cy*+*L*/*Cy*+*L* and +*Pm*+/+*Pm*+, are lethal. The *Cy*+*L*/+*Pm*+ flies form two kinds of gametes, *Cy*+*L* and +*Pm*+, re-

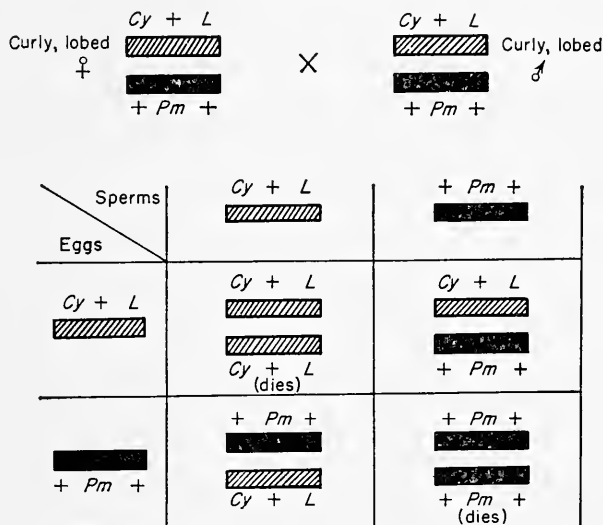


FIG. 12-12 Balanced lethal system in *Drosophila* for perpetuating hybrid condition. The homozygous *Cy*+*L*/*Cy*+*L* and +*Pm*+/+*Pm*+ flies die, leaving only heterozygotes.

sulting one fourth *Cy*+*L*/*Cy*+*L*, one half *Cy*+*L*/+*Pm*+, and one fourth +*Pm*+/+*Pm*+ individuals. Since the homozygous types die, only the heterozygous types, *Cy*+*L*/+*Pm*+, survive, giving a true breeding hybrid (Fig. 12-12). This balanced lethal stock has been useful in radiation experiments in determining all induced mutants in the second chromosome in *Drosophila*.

SELF-STERILITY ALLELES INSURE CROSS POLLINATION

An interesting series of self-sterility alleles insures cross pollination in several species of plants. The genetic explanation of this was determined in the genus *Nicotiana* by East and A. J. Mangelsdorf, one of East's graduate students. The operation of the self-sterility alleles is no more complicated than the operation of other genes, but detecting it represented a clever piece of genetic analysis.

It had been observed by several investigators that certain species of *Nicotiana* would set no seed if they were self-pollinated. Several of East's papers were concerned with this phenomenon. The explanation of East and Mangelsdorf proposed a series of self-sterility alleles, labeled $S_1, S_2, S_3 \dots S_n$. A plant could have any two of these, but no more, since they are alleles located opposite each other in a pair of chromosomes. Fertilization could be accomplished only by a pollen grain with one of the alleles not present. For example, S_1/S_2 plants if pollinated by S_1/S_2 pollen would set no seed, because neither S_1 nor S_2 could affect fertilization. Apparently, pollen tubes will not grow down a style of the same genotype. Various combinations of crosses with their progenies are shown in Table 12-1.

Table 12-1. Functional Pollen Produced and Progeny Resulting from Crosses of Different Genotypes of Self-Sterility Alleles^a

Genotypes of Parents	Functional Pollen ^a	Progeny
$S_1/S_2 \times \text{self}$	none	0 seed
$S_1/S_2 \times S_1/S_3$	S_3	$S_1/S_3 \quad S_2/S_3$
$S_1/S_2 \times S_2/S_3$	S_3	$S_1/S_3 \quad S_2/S_3$
$S_1/S_3 \times S_1/S_2$	S_2	$S_1/S_2 \quad S_2/S_3$
$S_1/S_3 \times S_2/S_3$	S_2	$S_1/S_2 \quad S_2/S_3$
$S_2/S_3 \times S_1/S_2$	S_1	$S_1/S_3 \quad S_1/S_2$
$S_2/S_3 \times S_1/S_3$	S_1	$S_1/S_2 \quad S_1/S_3$

^a See Fig. 12-13 for operation of these alleles.

East and Mangelsdorf found that self-sterile lines, which would normally set no seed if self-pollinated, could be made to set seed if the pollinations were made in the young bud. Self-pollination and crosses made in the bud produced the following results:

$$S_1/S_2 \text{ self-pollinated} \rightarrow \frac{1}{4} S_1/S_1 : \frac{1}{2} S_1/S_2 : \frac{1}{4} S_2/S_2.$$

The unusual types S_1/S_1 and S_2/S_2 , produced by bud pollination, gave the results illustrated in Table 12-2 when they were pollinated (open flowers) by flowers of different genotypes.

Table 12-2. Progeny of Unusual Plants Homozygous for Self-Sterility Alleles

Genotypes of Parents	Functional Pollen	Progeny
$S_1/S_1 \times S_2/S_3$	S_2, S_3	$S_1/S_2, S_1/S_3$
$S_1/S_1 \times S_1/S_3$	S_3	S_1/S_3
$S_1/S_1 \times S_1/S_2$	S_2	S_1/S_2
$S_2/S_2 \times S_1/S_2$	S_1	S_1/S_2
$S_2/S_2 \times S_1/S_3$	S_1, S_3	$S_1/S_2, S_2/S_3$
$S_2/S_2 \times S_2/S_3$	S_3	S_2/S_3

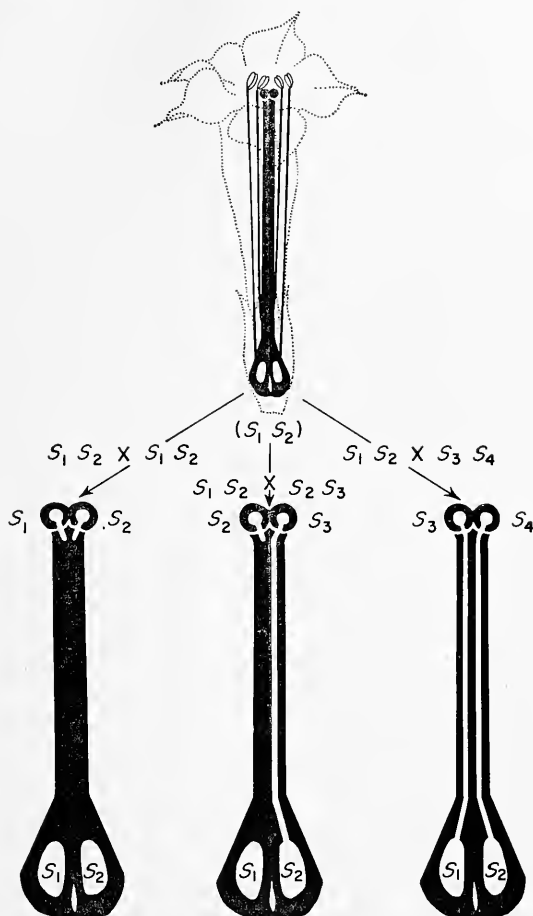


FIG. 12-13 Self-sterility alleles in *Nicotiana* for preventing self-pollination.

Although only three different alleles were postulated by East and Mangelsdorf, many more have been added to the list. They behave in a similar manner. The original three alleles explain adequately the working of self-sterility in *Nicotiana* and many other species. More complicated cases can now be found in many genetic publications.

OTHER METHODS OF PERPETUATING A HETEROZYGOUS CONDITION

In addition to the self-sterility alleles (or the balanced lethal systems) just described, there are other means of perpetuating a hybrid condition. Most of our varieties of horticultural plants, fruit trees, and shrubs are propagated asexually by cutting, budding, or grafting, or by new plants arising from

bulbs, tubers, runners, or the roots of the mother plant. The new plant has exactly the same chromosomal and genic complement, since there is no sexual reproduction with the concomitant shuffling and "dealing new hands" of the genetic material. Barring somatic mutation and disease, a strain can be reproduced indefinitely and any heterosis maintained.

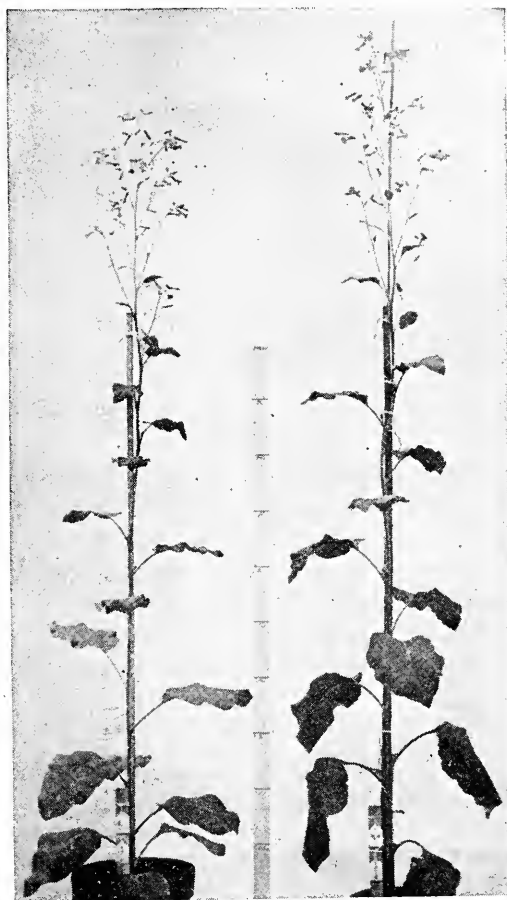
AMPHIDIPLOIDY—A FIXED HETEROSIS

An *amphidiploid* may be described as essentially a new species of plant produced from a hybrid by a process of chromosome doubling. There are many examples in the genetic literature. One of the first is the Raphanobrassica amphidiploid of Karpechenko (1928). He crossed a radish (*Raphanus sativus*) with a cabbage (*Brassica oleracea*) and obtained a vigorous, but almost completely sterile, F_1 hybrid. The haploid number of chromosomes of the two parental species is nine. The F_1 had a diploid number of 18. Nine of these were radish chromosomes, the other nine cabbage chromosomes. The few progeny from the F_1 were apparently produced by the union of an amphidiploid egg nucleus (18 chromosomes) with an amphidiploid sperm nucleus. They were amphidiploids with 18 radish chromosomes and 18 cabbage chromosomes.

The 18 pairs of chromosomes in the F_2 amphidiploid each had a partner so that meiosis could proceed normally, with concomitant restoration of fertility. The amphidiploid breeds true and constitutes a new genus of plant with a diphyletic origin.

AMPHIDIPLOID NICOTIANA

In the same year that Karpechenko reported his Raphanobrassica amphidiploid, we reported an analogous case of chromosome doubling in the genus *Nicotiana* (Singleton, 1928). Under East's direction, a cross was made between *Nicotiana rustica* and *N. paniculata*. The former species has 24 pairs of chromosomes, the latter 12. The cross can be made with ease if *N. rustica* is used as the female parent. The F_1 plants were vigorous and intermediate to the two parents in appearance. At meiosis they had 12 pairs and 12 single chromosomes (Fig. 12-14). The F_1 plants were only a fraction of a per cent fertile. It was quite surprising to find in the F_2 a high proportion of the plants with approximately 36 pairs of chromosomes, just twice the total complement of the F_1 . These plants resembled the F_1 in appearance, but were highly fertile when self-pollinated, or when pollinated by *N. rustica*. There was no set of seed when pollinated by *N. paniculata*. Here, then, was essentially a new species produced in the laboratory and completely isolated genetically from one of its parents, *N. paniculata*. It was true breeding and represented a fixation of heterosis and the type of the F_1 (Fig. 12-14).



Courtesy of Genetics

FIG. 12-14 Amphidiploid *N. rustica-paniculata* (right) in comparison with F_1 of *N. rustica* and *N. paniculata*.

BIOCHEMICAL BASIS OF HETEROSIS

In 1941, W. J. Robbins performed an interesting experiment that gave a clue to the mechanism underlying heterosis. He excised the roots of two varieties of tomatoes, Johannesfeuer and Red Currant. He grew these roots in comparison with the excised roots of the F_1 in liquid culture media, to which various vitamins were added. One parent, Johannesfeuer, showed a greater response to pyroxidin than did the other. Red Currant made a greater comparative response to supplements of nicotinamide. The hybrid, however, showed no beneficial response to the addition of either pyroxidin or nicotinamide. Apparently the hybrid was able to synthesize both of these products,

a result that can be given a genic interpretation. Let P represent the ability to synthesize pyroxidin, with the recessive p a lack of this ability. The letter N represents the ability to synthesize nicotinamide, with n the lack of it. The results of a cross between the two tomato varieties can be shown as follows:

Parents.....	Johannesfeuer	×	Red Currant
Genotype.....	N/N p/p	×	n/n P/P
F_1	N/n		P/p

The F_1 as illustrated was able to synthesize both vitamins, since it had one dominant allele at each locus. Such an F_1 should produce an F_2 with a 9:3:3:1 ratio for phenotypes $N/- P/-$, $N/- p/p$, $n/n P/-$, and $n/n p/p$. The $n/n p/p$ class should lack the ability to synthesize both nicotinamide and pyroxidin.

HETEROCARYOTIC VIGOR IN FUNGI

Neurospora, the organism that has been used so profitably to advance our knowledge of biochemical genetics, has also been studied to increase our understanding of heterosis. The mycelia of *Neurospora* are haploid, although they may have more than one nucleus. Should the nuclei all arise from a single nucleus they would be all of the same genotype and have no opportunity to show heterosis. However, mycelia arising from different nuclei may fuse, bringing together nuclei with different genotypes in the same mycelium. In such cases hybrid vigor is possible, even though the different genotypes are in different nuclei. Such a fusion is known as a *heterocaryon*. Beadle and Coonradt (1944) worked with two strains, *nicotiniless* and *pantothenicless*, each being dependent for growth upon the addition of either nicotinic acid or pantothenic acid. However, when a heterocaryon was formed between the two strains, it could grow in a medium lacking both nicotinic acid and pantothenic acid (Fig. 12-15).

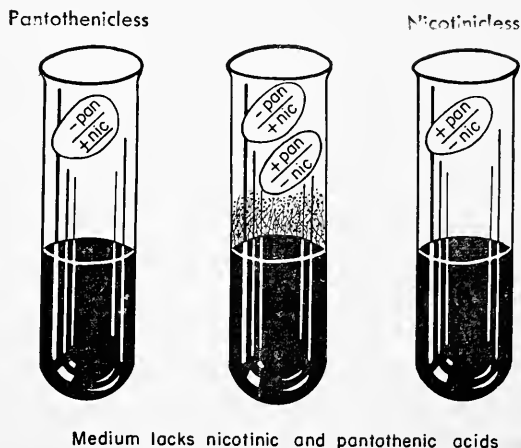


FIG. 12-15 Heterocaryon in *Neurospora*, producing hybrid vigor, or heterosis.

CONCLUDING REMARKS

The pure line concept of Johannsen has been explored. He found it of no value to select *within* pure lines of beans, but quite effective to select *among* lines, which was done in starting his experiment. Since beans are self-fertilized, all of his genotypes were pure lines. Shull, in corn, found essentially what Johannsen had found. Shull first had to isolate his pure lines, which he called biotypes. He and East found hybrid vigor when different inbred lines were crossed. This method is the basis of hybrid corn, using the pure-line method proposed by Shull, with Jones' modification called the double cross.

Controlled heredity is what has made hybrid corn so superior to the open-pollinated varieties. The hybrid vigor is due to the dominance of many different growth-promoting genes, either simple dominance or overdominance where the heterozygous condition A/a is superior to A/A . The inheritance of self-sterility alleles for preventing self-fertilization was shown, and also mechanisms for maintaining a constant heterozygous condition through balanced lethals, amphidiploidy, and asexual propagation.

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PROBLEMS

12-1. Define or describe the following:

amphidiploidy	inbred
asexual propagation	inbreeding
balanced lethal (<i>Drosophila</i>)	<i>N. rustica-paniculata</i>
balanced lethal (<i>Oenothera</i>)	amphidiploid
biochemical basis heterosis	nicotinicless <i>Neurospora</i>
biotype	overdominance
Curly-Lobe-Plum (<i>Drosophila</i>)	pantothenicless <i>Neurospora</i>
double cross (corn)	permanent heterosis in <i>Oenothera</i>
<i>gaudens</i> (<i>Oenothera</i>)	pure-line
<i>gaudens-velans</i> hybrid (<i>Oenothera</i>)	<i>Raphano-brassica</i> amphidiploid
genic explanation of hybrid vigor	selection among pure lines
heterocaryon	selection within pure lines
heterocaryotic vigor in fungi	selfing
heterosis	self-sterility alleles
hybrid corn	sib-pollination
hybrid vigor	single cross
	<i>velans</i> (<i>Oenothera</i>)

12-2. Identify the following scientists, giving a major contribution of each with an approximate date:

Beadle, G. W.	Jones, D. F.
East, E. M.	Mangelsdorf, A. J.
Emerson, R. A.	Robbins, W. J.
Hayes, H. K.	Shull, George H.
Johannsen, W.	

12-3. Suppose you are given two beans, weighing .35 cg and .55 cg, respectively. How would you determine whether these two beans represented a chance fluctuation from a single pure line, or two different pure lines?

12-4. In four o'clocks a cross of red by white produces pink flowers. Which color is dominant? What proportion of plants with pink flowers would there be in the following generations of a cross of white times red?

F_1 , F_2 , F_3 , F_4 , F_5

Give the formula for calculating the proportion of plants with pink flowers in any generation after being selfed n times.

12-5. Suppose you were given the following inbred lines of corn with different dominant growth promoting genes indicated by capital letters as follows:

Line 1	$A/A \ b/b \ C/C \ D/D \ e/e \ F/F$
Line 2	$A/A \ B/B \ C/C \ d/d \ e/e \ f/f$
Line 3	$A/A \ b/b \ C/C \ D/D \ e/e \ f/f$
Line 4	$a/a \ b/b \ c/c \ D/D \ E/E \ F/F$

Which two lines would you cross to produce a hybrid with maximum vigor? Why?

From four inbred lines, how many different possible crosses could you make? (Assume reciprocal hybrids would be the same.)

- 12-6. Suppose a cross were made between two self-sterile lines of *Nicotiana*, one of the genotypes S_1/S_2 and the other S_3/S_4 . Show by a diagram what progeny would be expected. If all of the progeny were crossed by an S_1/S_3 plant, show by the use of a diagram what the resulting progeny would be.

- 12-7. Suppose corn inbreds A and B had average yields of 48 and 52 bushels per acre respectively, while inbreds C and D averaged 37 and 43. The yields of the six possible single crosses of these 4 inbreds were as follows:

A·B 100, A·C 110, A·D 120

B·C 130, B·D 140, C·D 90

- a. What would be the expected yields of the two double crosses $A·B \times C·D$, and $A·C \times B·D$?

NOTE: The expected yields of the double cross $A·B \times C·D$ can be estimated by getting the average of the four single crosses A·C, A·D, B·C, and B·D.

- b. Which single crosses must be averaged to predict the yield of the double cross $A·C \times B·D$?
- c. Does it make a difference which way the four inbreds are combined to make the double cross?
- d. Would you expect the double cross to be more or less uniform than the single cross?
- 12-8. Explain why an amphidiploid such as the *Nicotiana rustica paniculata* F_2 should be almost completely fertile, whereas the F_1 was more than 99 per cent sterile. If you observed the size of pollen grains or stomata, how would those of the amphidiploid compare with those of its parents?
- 12-9. Explain how W. W. Robbins demonstrated the biochemical nature of heterosis in tomatoes.

Genetic Mechanisms for Sex Determination

FOLK TALES THROUGH THE AGES

Historically no progress was made in the genetics of sex determination before the sex chromosomes were found, soon after Mendel's laws were rediscovered. Lack of exact knowledge did not prevent speculation, as was pointed out by Conway Zirkle (1951), our most distinguished genetical historian. He said that there had been three fantastic beliefs regarding sex determination. One of these held that the fact of two testicles in the male was more than a coincidence. It was thought that the semen from one begot males, the other females. The right testis of course produced the dominant sex, the left one, females. This belief was prevalent among the ancient Hebrews. Actual attempts were made to alter sex ratios in flocks and herds by tying off one testicle before copulation. This belief persisted for centuries. Aristotle condemned it and said the reports of controlling sex by inactivating a testis were untrue and mere guesswork.

The second theory described by Zirkle was not subject to experimental testing and lasted even longer than the first. This asserted that the sex of the offspring was determined by whether the male or female parent was the more heavily sexed. An effeminate man could supposedly produce only daughters, a notion that has been exploited by many comedians. This legend endured even to the time of the discovery of the X and Y chromosomes. Even Darwin held that the embryo did not develop from a single egg impregnated by a single sperm, but was influenced by the whole mass of semen.

The third and most fanciful theory cited by Zirkle was that the sex of the offspring was determined by the direction the wind was blowing at the time of coition. If the north wind blew, males were engendered; if the south wind, females. Shepherds tried to take advantage of this by letting the rams in to the

ewes only when the south wind was blowing, so more females would be produced. According to Zirkle this notion was treated with respect by Aristotle and it persisted in publications down to the 17th Century.

JACOB'S BREEDING TECHNIQUE (GENESIS)

While we are on the subject of ancient history, it should be pointed out that the Biblical story of an animal-breeding scheme of Jacob (later named Israel) represents perhaps the first recorded attempt to change the genetic constitution of an animal population. Jacob devised a plan to cheat his father-in-law, Laban, out of his flock. Perhaps he was justified in doing this, because his father-in-law had made him work seven years in order to be eligible to marry Laban's attractive daughter, Rachel. At the end of seven years, however, Jacob received not Rachel but an older sister, Leah, to wed, and he had to work another seven years to obtain Rachel.

Jacob made a bargain with Laban that he would tend Laban's flocks, and for his compensation he should have as his own all the speckled cattle. Whereupon it seems Jacob proceeded to procure a "speckled" sire, in this case a dominant, and henceforth garnered in most of Laban's flock. Jacob explained his results by a cunning scheme of influencing the color of the animal by peeling poplar, almond, and plane (Sycamore) saplings and making a striped fence which he placed in front of the animals when they were bred. (Lysenko should admire this trick.) What is more important from a genetic standpoint is that he had a dream. "In the mating season of the flock I lifted up my eyes, and saw in a dream that the he-goats which leaped upon the flock were striped, spotted, and mottled." (*Genesis* 31:10.) Apparently, this was more than a dream, as is evidenced by the changing of the flock to "striped, speckled, and mottled."

JOHANN DZIERZON'S EXPERIMENTS WITH BEES

Zirkle gives Dzierzon credit for influencing Mendel in the manner in which he went about his experiments. Dzierzon bred honeybees. His work on the determination of sex in bees was far ahead of his time, just as were the experiments of Mendel. Dzierzon reported that drones were hatched from unfertilized eggs, but that workers and queens came from fertilized eggs. In the 1850's he published a number of papers and was well-known, at least to those who were interested in honeybees. His results gave rise to a violent controversy, which is more than can be said of Mendel's work, which was almost completely ignored at the time. In one of Dzierzon's experiments he crossed German with Italian bees and found that unmated hybrid queens produced German and Italian drones in equal numbers, a definite 1:1 ratio. The sex ratio of unmated females has been confirmed in bees and many wasps.

One of the wasps best studied genetically is *Habrobracon*. P. W. Whiting has given us a thorough explanation of sex determination in this organism, to be discussed later in this chapter.

Zirkle believed that Mendel knew of Dzierzon's work for three reasons: (1) Dzierzon was a well-known bee breeder, (2) Mendel also raised bees, and (3) Dzierzon was a fellow cleric who lived in nearby Silesia.

If, as Dzierzon found, the female honeybee produced two kinds of eggs in equal numbers, it must have seemed logical to him that the male would produce two kinds of sperm, also in equal numbers. Consequently, a ratio of 1:2:1 was inevitable, just as surely as $(a + b)^2$ equals $a^2 + 2ab + b^2$. If dominance existed, this would appear phenotypically as a 3:1 ratio. Zirkle believes that possibly Dzierzon's work alerted Mendel to the importance of definite ratios in interpreting his own results and in describing a possible mechanism of heredity. If so, the general importance of Dzierzon's brilliant work far exceeded his own specific studies.

X AND Y SEX CHROMOSOMES

In many animals, and in some dioecious plants, a heteromorphic pair of chromosomes has been identified with sex determination. In *Drosophila*, for example, the female has two X chromosomes, both alike, while the male has one X chromosome and another morphologically distinct, called a Y chromosome. In addition, there are three other pairs of chromosomes whose homologues are alike. Man has 23 pairs of chromosomes, 22 whose homologues are alike and one heteromorphic pair associated with sex determination. In some animals, particularly moths and birds, the chromosomes in the male are all paired perfectly, with no heteromorphic pair such as the X and Y chromosomes. In these cases the female has the heteromorphic pair of chromosomes. In poultry, the female has but one X chromosome with no mate. Consequently the female is known as one having an XO condition for the sex chromosomes, instead of the XY. She produces two kinds of gametes, one with an X, the other with none. The first gamete, with an X chromosome, produces a male when united with an X from the father, while the second produces a bird with the XO condition, a female. Consequently a 1:1 sex ratio is maintained.

Some plants that are dioecious [*di* Gr. *dis* twice + *iokos* house] have X and Y sex chromosomes, as found in animals. The pistillate plants have two X chromosomes; staminate plants an X and a Y. These two types are commonly referred to as females and males. Botanically speaking, this terminology is incorrect, since all plants are asexual, bearing spores. The pistillate plants produce megaspores which give rise to the gametophytic generation resulting in the female gamete, the egg nucleus. Likewise, staminate plants produce microspores (pollen grains), the gametophytic generation, giving rise to the

male gametes. The two sperm nuclei develop either in the pollen grain or in the pollen tube after the pollen grain has germinated on the stigma.

While the foregoing terminology is more precise, the student should be aware that pistillate plants are commonly referred to as "females" and the staminate ones as "males." The end result is the same: the pistillate (female) plants produce eggs, while the staminate (male) plants produce sperm. The only thing that makes this terminology improper is the short gametophytic generation described (occurring in plants, but not in animals), in which the females and males produce either eggs or sperm as the immediate result of meiosis. Functionally, pistillate and staminate plants are females and males, and many authors so designate them.

Usage generally determines the meaning of technical terms. Male and female are now accepted by Webster as equivalent to staminate and pistillate. Webster states, "In seed plants *male* is a popular equivalent of staminate." Many cases of gene-controlled sterility have been observed in plants. In some cases the plant has been rendered incapable of viable pollen production by the action of a specific gene. In maize more than a score of genes causing no pollen production have been recorded. These have been called "male sterile." To be botanically precise, they should be termed staminate sterile. However, all maize geneticists know them as male steriles, and the confusion would be greater than the gain in precision should an attempt be made to change the term.

"BALANCE" IN *DROSOPHILA* SEX DETERMINATION

In *Drosophila* the Y chromosome is mostly heterochromatic with no alleles for genes in the X chromosome. All genes in the X chromosomes show characteristic sex linked inheritance. The presence or absence of the Y sex chromosome is not the determining factor in sexual differentiation, but rather it is the balance of the number of X chromosomes and the number of sets of autosomes present.

The balance theory of sex determination was proposed by Calvin B. Bridges (1925). His work began with an accidental triploid ($3n$) *Drosophila* (with three X chromosomes and three sets of autosomes). He crossed the triploid ($3n$) female with a normal male and secured a number of interesting individuals, which differed in the number of X chromosomes and the number of sets of autosomes. Bridges described a "set" of autosomes as consisting of a second and a third chromosome; the small fourth chromosome could be present singly or in duplicate. The results of Bridges' experiment are shown in Table 13-1, with the ratio of X chromosomes to autosomes. In a normal female with two X chromosomes and two sets of autosomes the ratio of X chromosomes to autosomes is one. In a normal male the ratio of X chromosomes to autosomes is one half, since there is but one X chromosome and two sets of autosomes.

Table 13-1. Balance of X Chromosomes and Autosomes (Nos. 2 and 3)
in *Drosophila* Chromosomal Constitution (data of Bridges)

	Chromosome 1		Autosomes 2 and 3	Chromosome 4	Ratio X/A	Phenotype
	X	Y				
(1)	XX	—	2 sets	2	1.0	normal female
(2)	X	Y	2 sets	2	.5	normal male
(3)	XXX	—	3 sets	3	1.0	triploid female

Kinds of Progeny Produced by Crossing 3 × 2

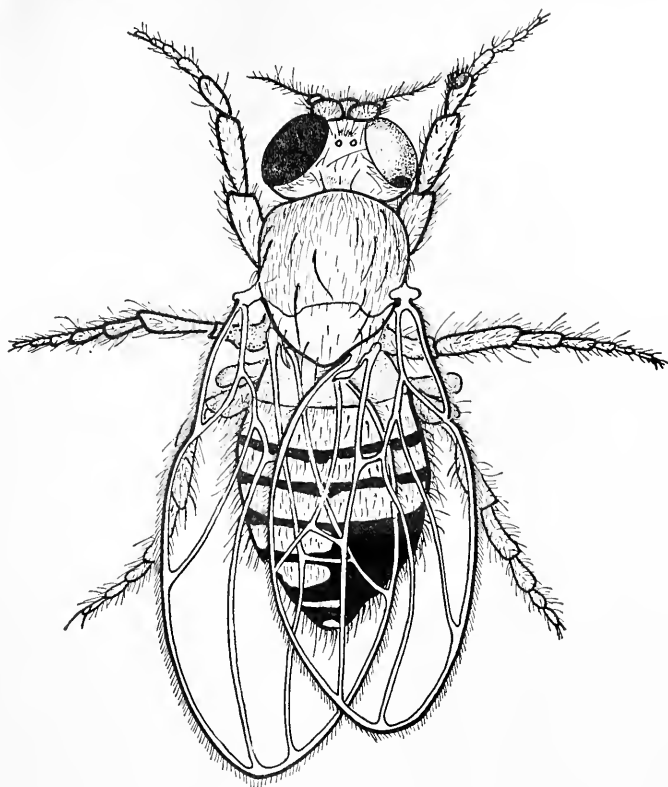
(4)	XXX	—	3 sets	1 or 2	1.0	triploid female
(5)	XX	—	2 sets	2	1.0	diploid female
(6)	XX	Y	2 sets	2	1.0	diploid female
(7)	XX	—	3 sets	1 or 2	.67	intersex
(8)	XX	Y	3 sets	1 or 2	.67	intersex
(9)	X	Y	2 sets	1 or 2	.50	normal male
(10)	XXX	—	2 sets	1 or 2	1.5	superfemale
(11)	X	Y	3 sets	1 or 2	.33	supermale

Intersexes are sterile flies intermediate between males and females. They are not to be confused with *gynandromorphs*, which have part of the body female and the rest male. Gynandromorphs have given us valuable information on the correlation of genetics and cytology.

The “superfemales” and “supermales” classes, 10 and 11, are not really over-sexed individuals, as the name might imply. Rather they are sterile individuals that show only minor phenotypic differences from ordinary males and females.

GYNANDROMORPHS IN DROSOPHILA

An excellent place to observe the phenotypic differences between males and females is in an occasional *Drosophila* gynandromorph [Gr. *gyn*—female, and *andro*—male], a combination of a female and male. Different parts of the body are phenotypically either male or female, not a mixture of the two. Morgan and Bridges (1919) obtained a gynandromorph with one half of the body male, and the other half female. Fortunately this fly was heterozygous for white eye and miniature wing, two sex-linked characters. The genotype was *w m/+ +*. In the gynandromorph one half was female and *++*, as if it still had both X chromosomes, while the other half was white-eyed, miniature-winged and male in appearance. Apparently this half had lost the X chromosome carrying the two dominant alleles, and now had only the



After Bridges

FIG. 13-1 Gynandromorph in *Drosophila*, heterozygous for $+/w m$ in X chromosome. The female half has dominant alleles of w and m , while the male half is white-eyed and has a miniature body. Apparently the male half has but one X chromosome (hemizygous), having lost one, most likely by non-disjunction at first division in the embryo.

one X chromosome with the alleles w and m in the hemizygous state (Fig. 13-1).

SEX CHROMOSOMES IN MAN

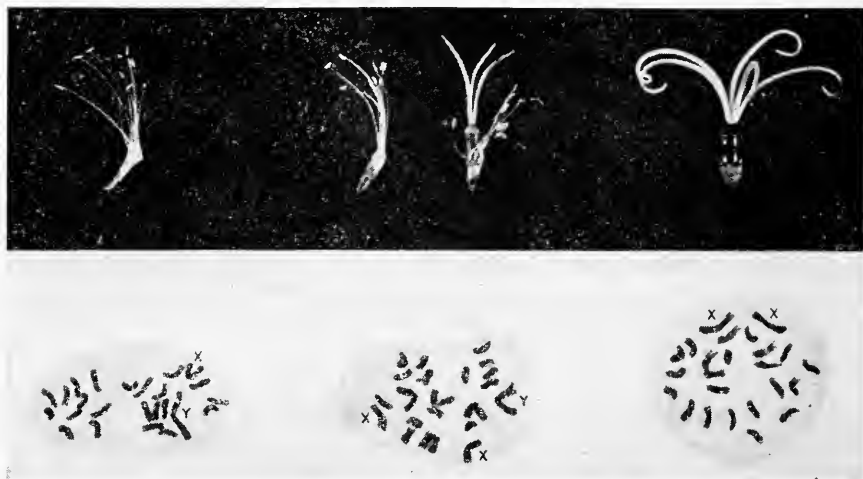
The situation regarding sex chromosomes in man is similar to that in *Drosophila*. XX individuals are females, XY males. In man, however, the Y chromosome apparently is not all inert, as in *Drosophila*. Recently, cytological observations of human beings have revealed individuals that differ from the normal XX and XY genotypes. For example, Turner's syndrome has been analyzed cytologically and found to be XO in genotype, with only one X chromosome and no Y. This syndrome results in stunted growth of

both size and weight and in malformed digits. What is important to the theory of sex determination is that the individual is a phenotypic female with rudimentary sex organs. (In *Drosophila* XO individuals are phenotypic males, since the Y chromosome is without effect.) Apparently, the Y chromosome in man carries some male-promoting genes; hence an XO individual is a phenotypic female because of the lack of the Y chromosome.

Another piece of evidence for the influence of the Y chromosome is Klinefelter's syndrome, which is XXY, a phenotypic male. In man the Y chromosome is important in sex determination. This emphasizes the fact that the same mechanism is not applicable to all organisms. Also in man there are known genes in a portion of the Y chromosome homologous with a portion of X.

SEX DETERMINATION IN MELANDRIUM

In *Melandrium*, a dioecious genus in the pink family (a variety of garden flower), sex is determined by a pair of XY chromosomes, just as it is in many animals. There are 12 pairs of chromosomes in the normal diploid, 11 pairs of autosomes plus an X and Y. The X and Y are quite different



Courtesy of H. E. Warmke; and American Journal of Botany

FIG. 13-2 Staminate (male) blossom in *Melandrium*, with XY sex chromosomes (left). The pistillate (female) blossom with XX chromosomes (right). Male flower with an XXY genotype (center).

morphologically (Fig. 13-2). Females or pistillate plants are XX, staminate XY. A pistillate plant is determined by a lack of the Y chromosome rather than by the two X chromosomes.

By using colchicine to induce polyploidy, it has been possible to obtain a series of plants differing markedly in their chromosome numbers (Warmke,

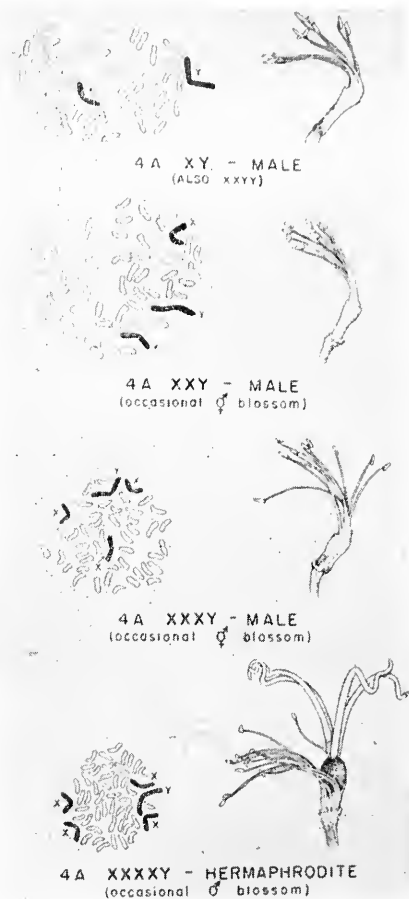
1946). Warmke found that the Y chromosome is most important in sex determination. In fact, one Y chromosome is equal to approximately four X chromosomes in determining sex (Fig. 13-3). In *Melandrium* the balance is between the X and the Y, with the number of autosomes having no effect, in contrast to the situation in *Drosophila*. This is shown in Tables 13-2 and 13-3 and is illustrated in Figs. 13-4 and 13-5.

In the examples just discussed, we have seen that sex is determined by a balance between the X chromosomes and the autosomes (*Drosophila*), or by the influence of the Y chromosome (man and *Melandrium*). Apparently in *Drosophila* the autosomes contain male-promoting genes which are balanced against the female-promoting genes in the X chromosomes. In man and *Melandrium* the X chromosomes contain female-promoting genes (similar to *Drosophila*). However, the male-promoting genes are in the Y chromosome, in contrast to *Drosophila*.

TRANSFORMER GENE (*TRA*) AND PHENOTYPIC SEX- UAL EXPRESSION IN *DROSOPHILA*

In *Drosophila* an autosomal, recessive, transformer gene, when homozygous, acts upon normal females to covert them into phenotypic males that are sterile. Normal X/Y males may be homozygous *tra/tra* and are fertile. If a normal fertile female is crossed by a *tra/tra* male the results are as shown in Table 13-4.

In the F_2 a ratio of five phenotypic males to three females is obtained instead of the customary 1:1 ratio. In the testcross, a 3:1 ratio of males to females is obtained, instead of the conventional 1:1 ratio expected for such a mating.

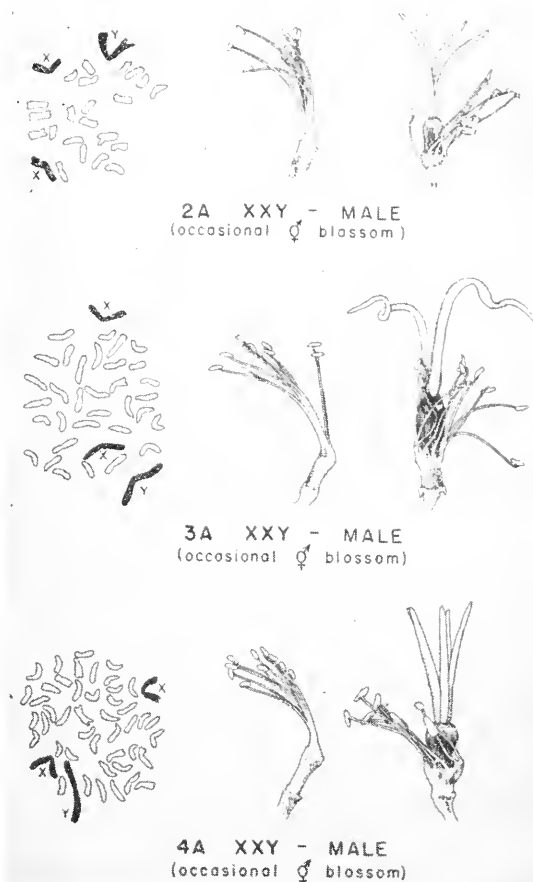


Courtesy of H. E. Warmke; and
American Journal of Botany

FIG. 13-3 Male blossoms in *Melandrium* have at least one Y chromosome and may have up to four X chromosomes, which shows strong "male-promoting" genes in Y chromosome.

Table 13-2. Balance of X and Y Chromosomes in Sex Determination in *Melandrium* (after Warmke)

No. sets autosomes	XY Complement	Sex of plant
2A	XX	♀ normal pistillate plant, no anthers
2A	XY	♂ normal staminate plant, anthers, pollen
2A	XXY	♂ plant, occasional hermaphroditic blossom
3A	XXY	♂ plant, occasional hermaphroditic blossom
4A	XXY	♂ plant, occasional hermaphroditic blossom
4A	XY	♂ plant, staminate
4A	XXX	♂ plant, occasional hermaphroditic blossom
4A	XXXXY	— hermaphrodite, occasional staminate blossom

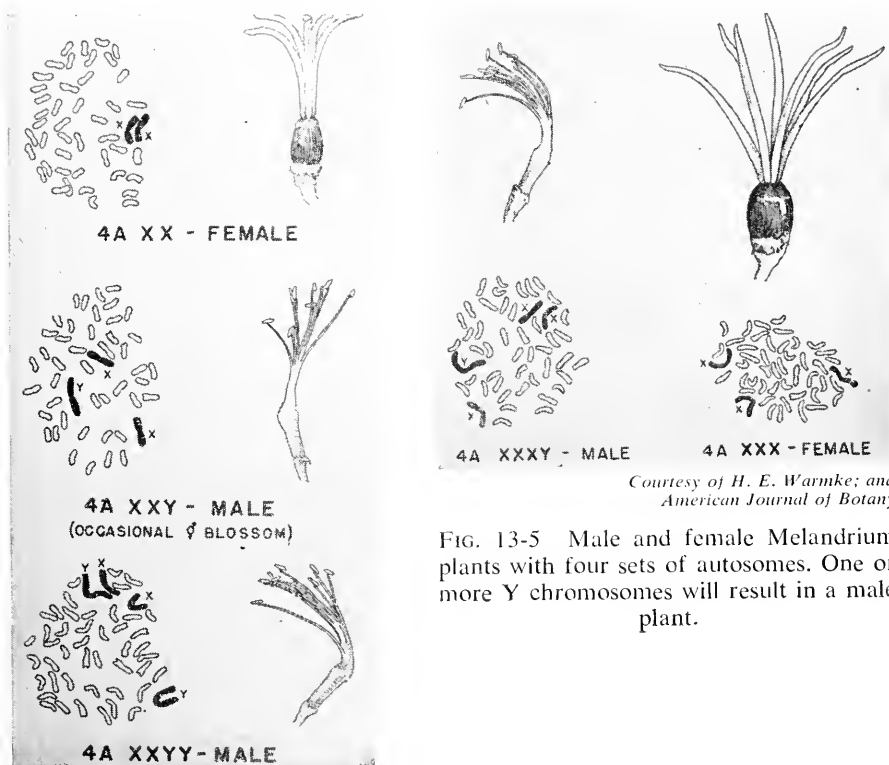


Courtesy of H. E. Warmke; and American Journal of Botany

FIG. 13-4 Number of sets of autosomes in *Melandrium* has no effect on sex determination. Specimen XXY is a male with two, three, and four sets of autosomes.

Table 13-3. Lack of Relationship Between X Chromosomes and Autosomes in *Melandrium* (after Warmke)

Autosomes	X Chromosome Constitution	X/A Ratio	Sex
4A	XX	0.50	pistillate (female)
3A	XX	0.67	pistillate (female)
4A	XXX	0.75	pistillate (female)
2A	XX	1.0	pistillate (female)
3A	XXX	1.0	pistillate (female)
4A	XXXX	1.0	pistillate (female)
4A	XXXXX	1.25	pistillate (female)
2A	XXX	1.50	pistillate (female)



Courtesy of H. E. Warmke; and
American Journal of Botany

FIG. 13-5 Male and female *Melandrium* plants with four sets of autosomes. One or more Y chromosomes will result in a male plant.

Table 13-4. Parents, F_1 , F_2 , and Testcross of Normal Female and Male Homozygous for Transformer Gene (*tra*)

		P							
X/X	+ / + ♀	×	X/Y	tra/tra ♂					
		F ₁							
X/X	+ / tra ♀	×	X/Y	+ / tra ♂					
		F ₂							
♀ gametes	♂ gametes	X	+	X	tra	Y	+	Y	tra
X	+	X/X female	+ / +	X/X female	+ / tra	X/Y male	+ / +	X/Y male	+ / tra
X	tra	X/X female	+ / tra	X/X "transformed" sterile male	tra / tra	X/Y male	+ / tra	X/Y male	tra / tra

Testcross

♀ gametes	♂ gametes	X	tra	Y	tra
X	+	X/X	+/tra	X/Y	+/tra
		female		male	
X	tra	X/X	tra/tra	X/Y	tra/tra
		"transformed"		male	
		sterile male			

GENE FOR SEX EXPRESSION IN MAIZE

The corn plant is normally monoecious, with both the staminate inflorescence (tassel) and the pistillate (ear) located in the same plant. Apparently a large number of normal sex-promoting genes are distributed throughout

the ten chromosomes. The result is that the sex of the plant is kept in balance, with the ear normally bearing seeds and the tassel producing pollen.

Many geneticists in their studies have observed mutations from the normal. For example, more than two score mutants for male sterile have been recorded. The first was male sterile-1 (ms_1) located in chromosome 6, described by Singleton and Jones (1930). The tassel produces no pollen (Fig. 13-6).



Courtesy of Connecticut Agricultural Experiment Station, New Haven

FIG. 13-6 Normal tassel in maize (left) with the more slender tassel of male sterile plants ms_1 . The anthers fail to extrude and contain no pollen.

Phenotypically the other different male steriles are similar to ms_1 . Another gene that prevents pollen formation is tassel seed, in which silks (pistils), instead of stamens, are produced in the tassel. Five different tassel-seed types were described in 1935 (Emerson, Beadle, and Fraser). An example of this is shown in Fig. 13-7. Both the male sterile genes and the tassel seed genes render the plant incapable of pollen formation, effectively making these genetic types pistillate or "female" plants.

Another gene, silkless, acts in the opposite direction, preventing silks (pistils) from being formed. Homozygous sk/sk plants produce "ears" which are nothing more than cobs, since there are no silks (pistils) (Fig. 13-8). This plant is effectively a staminate one, a "male" type. It must be maintained in the heterozygous condition.

MAIZE CHANGED FROM MONOECIUS TO DIOECIOUS TYPE

Since there are genes that change corn plants from a monoecious to staminate and pistillate types, Jones wondered whether it would not be possible to change corn from a monoecious to a dioecious type, one that



FIG. 13-7 "Tassel seed" tassel in maize which produces both silks and seeds, instead of pollen only in the tassel, a female type. Silkless at right.

Table 13-5. Parents, F_1 , and F_2 in Mating of Tassel Seed by Silkless Maize

P			
(tassel seed) ts/ts $+/+$		\times	$+/+$ sk/sk (silkless)
F_1			
$ts/+$	$+/sk$ (normal silks and tassel)		
F_2			
Phenotypic ratio		Phenotype	
9 $+/+$	$+/+$	normal tassels, silks	
3 ts/ts	$+/+$	tassel seed, normal silks	
3 $+/+$	sk/sk	normal tassel, silkless	
1 ts/ts	sk/sk	?	?

would segregate into equal numbers of pistillate and staminate plants. Such a segregation suggested a testcross ratio. He crossed a tassel-seed genotype by a silkless one. The results are diagrammed in Table 13-5.

At the time that the experiment began, it was not known what would be the phenotype of the double recessive. There were two clues as to the identity of the double recessive phenotype:



Courtesy of Connecticut Agricultural Experiment Station, New Haven

FIG. 13-8 Silkless maize ears, a male type. No silks are produced, consequently no kernels.

1. The ratio instead of being a 9:3:3:1 was a 9:3:4 for normal, silkless, and tassel seed, indicating that the double recessive homozygous class was phenotypically the same as the $ts/ts + /-$ class.

2. Some of the F_2 tassel-seed types when crossed by normal silkless $+/+ sk/sk$, produced progenies with 100% silkless plants.

The second could occur only if sk/sk plants were pollinated by sk/sk . It would be impossible with the regular sk/sk stock, since no silks are produced. Under the influence of ts/ts , however, sk/sk plants produce silks in the ear as well as in the tassel. The female-promoting tendency of the ts/ts stock completely "overpowers" the male-promoting tendency of the sk/sk stock.

This is an excellent example of the way one gene can completely subdue the effect of another of the opposite tendency. Thus ts/ts can be said to be epistatic to sk/sk .

Genes for expression of sex are located in several, perhaps all, of the 10 chromosomes of maize, which in a real sense are all autosomes, since there are no sex chromosomes (XY) in maize.

The final product of the dioecious maize created by Jones was as follows:

Female type $ts/ts\ sk/sk$ Male type $+/ts\ sk/sk$

These types are produced in equal numbers, a testercross ratio since only one gene pair is segregating, the other being homozygous recessive (Table 13-6).

Table 13-6. Mating Types Necessary to Produce Dioecious Maize
($ts/ts\ sk/sk\ ♀ \times +\ ts\ sk/sk\ ♂$)

♀ gametes ↙ ↘	♂ gametes		
	+	sk	
ts sk (only one type)	$+/ts\ sk/sk$ staminate (male)	$ts/ts\ sk/sk$ pistillate (female)	

SEX EXPRESSION IN ASPARAGUS

Normally asparagus is a dioecious species. Staminate and pistillate plants occur in approximately equal numbers. There is a slight tendency toward perfect flowers, in that normal pistillate flowers have rudimentary anthers, while staminate flowers sometimes have rudimentary non-functional pistils. Very rarely seeds are produced on staminate flowers. C. M. Rick and C. G. Hanna at the University of California found one such staminate plant that produced viable seeds, most likely self-pollinated. The seeds produced 155 staminate ($P/-$) and 43 pistillate plants p/p . This is close to a 3:1 ratio, suggesting a monogenic segregation.

Pollen from some of the 155 staminate plants was used to pollinate normal pistillate plants. Approximately one third bred true for staminate, while two thirds segregated into a 1:1 ratio for pistillate to staminate.

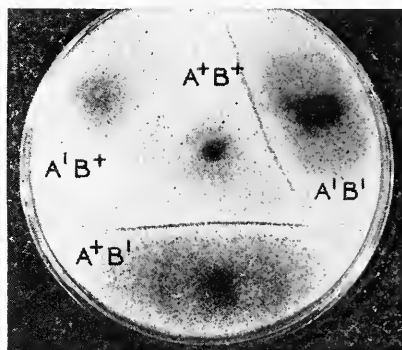
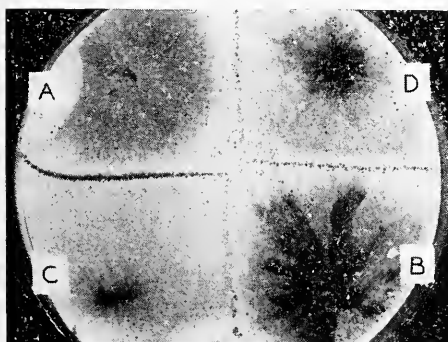
Assigning gene symbols to the types described above, a diagram of the results is shown in Table 13-7.

Table 13-7. Breeding Behavior of Unusual Staminate Plant (P/p) in *Asparagus*

(F ₁)	
P/p original plant (staminate)	
F ₂	
Observed 155 $P/-$	43 p/p
Expected 148.5	49.5
Expected F ₂ genotypes of 155 $P/-$	
(52) $\frac{1}{3} P/P \times p/p$	all staminate P/p
(103) $\frac{2}{3} P/p \times p/p$	1 P/p staminate 1 p/p pistillate

PREFERENTIAL MATING TYPES IN GLOMERELLA

Not always is there such a distinct manifestation of individual genes for sexual expression as the examples just cited, which gave characteristic results regardless of the other genes present. More complicated arrangements exist in some of the microorganisms that have different mating types. An example is *Glomerella*, a plant pathogen, which is an Ascomycete, one of a group of fungi including *Neurospora*. Although nearly all perithecial strains are fertile within themselves and will mate with most others, they show marked preferences for certain strains. This was demonstrated by C. L. Markert, who placed four strains of *Glomerella* on a petri dish and allowed them to grow and be cross-fertilized (Fig. 13-9 left).



Courtesy of C. L. Markert; and *American Naturalist*

FIG. 13-9 (Left) Comparative fertility of different strains of *Glomerella* when mated together. The petri dish is divided into quadrants by lines of perithecia, representing matings between different strains. (Right) Control of mating reaction by two pairs of allelic genes A^+/A^1 and B^+/B^1 . A^+ controls formation of perithecia, A^1 conidia formation. B^+ produces wide distribution, B^1 concentrated fruiting structures.

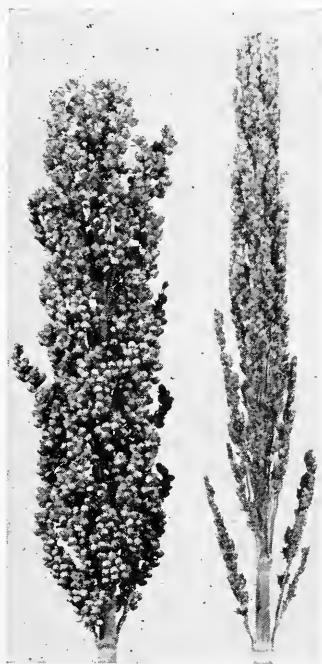
The lines dividing the petri dish into quadrants (13-9 left) are fruiting bodies, perithecia, formed where two different mating types came together. It can be seen that there is no one predominant "sex" when compared to all the others, but that some prefer a particular mating type. For example, there is a marked attraction of A for C, as shown by the heavy line of perithecia between them, but A does not have an equal preference for D, nor B for C. The various strains differ markedly in attraction for other mating types. The letters A, B, C, and D are arbitrary designations of different strains of *Glomerella*.

Different genes control mating reactions as shown in Fig. 13-9 right. There are two pairs of genes involved with four alleles A^+ , A^1 , B^+ and B^1 . The A^+ allele controls the formation of perithecia while the A^1 allele determines conidia formation. The alleles at the B locus control the distribution of the

reproductive structures. The strains A and B in Fig. 13-9 left are perithecial strains, i.e., with the A^+ allele, while the strains C and D are conidial strains with the A^1 allele.

COMPLEMENTARY GENES FOR FEMALE STERILE IN SORGHUM

An interesting case of female sterility in sorghum recently has been reported (Casady, Heyne and Weibel, 1960). The action of two complementary genes in the heterozygote produces a plant that is female sterile, although producing ample pollen. This is analogous to the silkless gene in maize (a recessive sk/sk that is essentially a female sterile). The female sterile in sorghum (Fig. 13-10) occurs when both dominant genes are heterozygous $Fs_1/fs_1 Fs_2/fs_2$. When three dominant genes are present, $Fs_1/Fs_1 Fs_2/fs_2$ or $Fs_1/fs_1 Fs_2/Fs_2$, the phenotype is a dwarf plant without any head being formed. An individual homozygous for both Fs genes, i.e., $Fs_1/Fs_1 Fs_2/Fs_2$, was not recovered. It is assumed that it would also be a headless dwarf producing no seeds. The genes Fs_1 and Fs_2 have no effect when occurring alone, either homozygous or heterozygous.



Courtesy of A. J. Casady,
E. G. Heyne, and D. E. Weibel;
and *Journal of Heredity*

FIG. 13-10 Normal head of sorghum (left) with female sterile head.

SEX DETERMINATION IN HABROBRACON

In the Hymenoptera, which includes wasps, bees, and ants, there is an unusual method of sex determination. Unfertilized eggs of females give rise parthenogenetically to haploid offspring, which are males. Females arise from fertilized eggs, which are diploid. For example, in bees the queen and the workers are diploid, while the drone is haploid. The haploid male produces haploid sperm which unite with haploid eggs of the female to produce queens and workers.

Sex determination in *Habrobracon* follows a similar pattern with something new added: diploid males. As in bees, the fertilized eggs become females, while unfertilized eggs become males. Also, some fertilized eggs become males, as has been shown by Phineas W. Whiting, who worked out the sex determination in this organism.

In addition to the regular pattern of sex determination in other Hymenoptera just described, there are a number of alleles promoting maleness or femaleness. These alleles act in a complementary fashion to produce females, which may be heterozygous for any two alleles. The males have only one of these alleles. They have been labeled by Whiting *xa*, *xb*, *xc*, *xd*, etc. A haploid male could be either *xa*, *xb*, *xc*, *xd*, etc., while the diploid male would be *xa/xa*, *xb/xb*, *xc/xc*, or *xd/d*. In either case only one kind of sperm could be produced by any given male. The female, on the other hand, is heterozygous for the sex-determining alleles. She could be *xa/xb*, *xa/xc*, *xa/xd*, *xb/xc*, *xb/xd*, and *xc/xd*, if only four of these alleles were concerned. Matings of the different kinds of females with one type of males *xa*, or *xa/xa*, are shown in Table 13-8.

Table 13-8. Matings of Different Genotypes of *Habrobracon* Females by *xa* Male

Genotype of female	Unfertilized eggs → haploid males. About 25% of progeny		Eggs fertilized by <i>xa</i> sperm. About 75% of progeny	
	Genotype		Genotype of Diploid Progeny	
<i>xa/xb</i>	<i>xa</i>	<i>xb</i>	<i>xa/xa</i> ♂	<i>xb/xa</i> ♀
<i>xa/xc</i>	<i>xa</i>	<i>xc</i>	<i>xa/xa</i> ♂	<i>xc/xa</i> ♀
<i>xa/xd</i>	<i>xa</i>	<i>xd</i>	<i>xa/xa</i> ♂	<i>xd/xa</i> ♀
<i>xb/xc</i>	<i>xb</i>	<i>xc</i>	<i>xb/xa</i> ♀	<i>xc/xa</i> ♀
<i>xb/xd</i>	<i>xb</i>	<i>xd</i>	<i>xb/xa</i> ♀	<i>xd/xa</i> ♀
<i>xc/xd</i>	<i>xc</i>	<i>xd</i>	<i>xc/xa</i> ♀	<i>xd/xa</i> ♀

The table gives all the possibilities of mating the different types of females by either a haploid or a diploid male bearing the *xa* allele for sex determination. The male produces but one type of sperm, while the female is heterozygous for the sex-determining alleles.

SEX IN BACTERIA AND BACTERIOPHAGE

Not only in higher plants and animals is there segregation for sex-determining factors, but also in the lower forms. Different mating types have been discussed in *Neurospora* and *Glomerella*. Tatum and one of his graduate students, J. Lederberg, at Yale University, first demonstrated recombination in bacteria, which could result only from some kind of sexual phenomena. For this brilliant work and for previous work of Tatum with Beadle, all three of these investigators were awarded the Nobel Prizes in Medicine in 1958.

Sexual recombination in bacteriophage (Fig. 13-11) was demonstrated by Delbrück and Delbrück in 1948. When two types of bacteriophage were added to a culture of bacterium sensitive to both, and allowed to incubate for a short period, it was found that the parental types were recovered. Also, two new types, which represented recombinations of the parental characteristics, were seen. If we represent the parents by Ab and aB, the recovered types

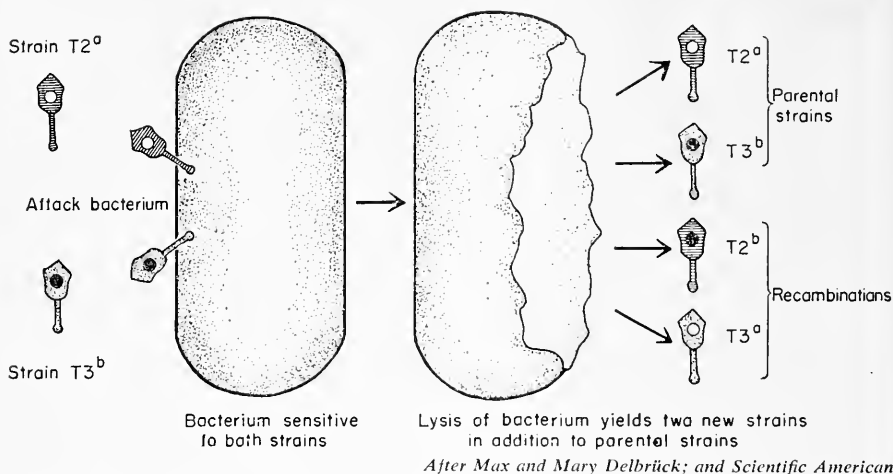


FIG. 13-11 Sexual recombination in bacteriophage. The parental types plus two new types are recovered, suggesting sexual reproduction.

include both *Ab* and *aB* and also *AB* and *ab* as well. The designations familiar to all phage geneticists are *T2* and *T4^r*. Both *T2* and *T4^r* were recovered, also *T2^r* and *T4*. In Fig. 13-11 these are labeled *T2^a* and *T3^b*, with the crossovers *T2^b* and *T3^a*. The labeling of the different types is relatively unimportant. What is important is the fact that sexual recombinations occur in bacteriophage, the smallest living organism.

MATING TYPES IN PARAMECIUM

Paramecium has been shown to have a number of mating types similar to some of the lowest forms of plant life (Sonneborn, 1947). *Paramecium* has two nuclei. One is a vegetative macronucleus that divides amitotically and is of no concern in regard to sex determination. The other is a micronucleus which is genetically active. It divides mitotically in *paramecia* that reproduce asexually. They may undergo many generations asexually and only conjugate when brought together with a different mating type. There may be as many as eight of these mating types, or eight sexes, in contrast to the two found in higher animals and plants. When conjugation takes place, the macronuclei degenerate while the micronuclei undergo the usual two meiotic divisions. Three of the daughter nuclei then degenerate. The fourth divides mitotically to form both a stationary nucleus and a wandering nucleus in each member of the conjugating pair. The wandering nucleus then migrates into the other conjugant, establishing a mutual cross-fertilization. The conjugants then separate. Their diploid nuclei divide to form a micronucleus and a vegetative macronucleus. More will be said about *paramecium* in Chapter 15 when discussing extranuclear inheritance.

ENVIRONMENTAL DETERMINATION OF SEX

An example of the influence of environment upon sex determination occurs in the marine worm *Bonellia*. The females are about an inch long, while the males are very tiny and live as parasites upon the female.

The eggs of *Bonellia* develop into larvae, sexually undifferentiated. If they settle down upon the substrate by themselves, they become females. Those that settle upon the proboscis of the females become males, a convenient arrangement for assuring fertilization. Apparently the female produces a substance that causes the attached larva to develop into a male. If incompletely differentiated males are separated from the controlling female, intersexes result.

CONCLUDING REMARKS

The historical importance of Dzierzon's work on the study of sex in bees, which probably had an influence on Mendel has been shown in this chapter.

The significance of the X and Y chromosomes in the determination of the sexes was presented. In *Drosophila*, differentiation of sex is dependent upon the balance between the number of X chromosomes and the number of sets of autosomes, with the Y chromosome being essentially inert. In man, the Y chromosome is a determining factor.

In some dioecious plants, such as *Melandrium*, the Y chromosome is very important, one Y chromosome being equivalent to four X chromosomes. Autosomes are without effect in sex determination.

Several cases of gene-controlled sexual sterility in plants were shown. Many genes causing male sterile are known. Male sterile is used synonymously with staminate sterile. In practice, "male" and "female" in plants are used interchangeably with staminate and pistillate, more precise terms botanically, but not in common usage among plant geneticists.

An unusual type of sexual differentiation occurs in some of the Hymenoptera, especially bees and wasps, in which haploid eggs develop parthenogenetically into males, while fertilized eggs produce females. In the parasitic wasp, *Habrobracon*, males are also produced from fertilized eggs.

Sexual differentiation is found in the lower forms of plant and animal life even to the smallest forms, the bacteriophage. We have also discussed the unusual example of *Bonellia*, in which the environment plays a part in sexual determination.

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PROBLEMS

13-1. Define or describe the following:

autosome	male-sterile (maize)
bacteriophage	megaspore
balance in <i>Drosophila</i> sex determination	microspore
dioecious	monoecious
environmental determination of sex	<i>Paramecium</i>
epistatic	pistillate
female plant	preferential mating (<i>Glomerella</i>)
female-sterility (sorghum)	sex chromosome
<i>Glomerella</i>	sex determination in <i>Paramecium</i>
gynandromorph	sex promoting alleles (<i>Habrobracon</i>)
<i>Habrobracon</i>	silkless maize
heteromorphic chromosomes	staminate
intersex	super female (<i>Drosophila</i>)
Klinefelter's syndrome (man)	super male (<i>Drosophila</i>)
male plant	tassel seed maize
	transformer gene <i>tra</i> (<i>Drosophila</i>)
	Turner's syndrome (man)

13-2. Identify the following scientists, giving a major contribution of each, with an approximate date.

Bridges, C. B.	Jones, D. F.
Dzierzon, J.	Lederberg, J.
Delbrück, Max	Markert, C. L.

Morgan, T. H.
Rick, C. M.
Sonneborn, T. M.
Tatum, E. L.

Warmke, H. E.
Whiting, P. W.
Zirkle, C.

13-3. Tell what sex the following *Drosophila* would be:

No. sets autosomes	X/Y composition
2	XX
2	XY
2	XXY
3	XX
3	XY
2	XO
2	XXX

13-4. Tell what sex the following *Melandrium* plants would be:

No. sets autosomes	X/Y composition
2	XX
2	XY
3	XY
4	XY
2	XXY
2	XXX
2	XXXXY
3	XXY
3	XXX

13-5. In *Habrobracon*, sex is determined in two ways. Parthenogenetic haploid individuals are always males. Also some diploid individuals are males if homozygous for sex-determining alleles. These alleles have been labeled *xa*, *xb*, *xc*, etc. Approximately 25 per cent of the eggs of a female are unfertilized and produce haploid males. What would be the percentage of males and females in the following crosses?

Cross	Per cent ♂	Per cent ♀
<i>xa/xb</i> ♀ × <i>xa</i> ♂		
<i>xa/xb</i> ♀ × <i>xc</i> ♂		
<i>xa/xc</i> ♀ × <i>xc</i> ♂		
<i>xb/xc</i> ♀ × <i>xb</i> ♂		

13-6. In *Habrobracon* the gene for fused (*fu*) is in the same chromosome as the alleles for sex determination, *xa*, *xb*, *xc*, etc., with approximately 10 per cent crossing-over between *fu* and the *xa*, *xb*, *xc* locus. A cross of an *xa fu/xb+* ♀ by an *xb fu* ♂ would give the ratios in the following table:

Offspring	Fused	Non-fused
Female		
Diploid male		
Haploid male		

13-7. The gene for veinless wing (*vl*) is an autosomal recessive, not in the same chromosome as *xa*, *xb*, and *xc*. How could this gene and its dominant allele be utilized in making crosses so that haploid males could be distinguished from diploid males?

13-8. In corn the recessive gene for barren stalk *ba* causes no ear to be developed in homozygous recessive plants *ba/ba*. Design an experiment to change

corn to a dioecious species, using this gene and tassel seed, a pistillate type (*ts/ts*).

- 13.9. A case of sex reversal in chickens has occurred in which a female was changed to a functional male which still had the chromosomal constitution of a female. What ratio of males to females would result from mating this "male" to a normal female? (A chicken must possess at least one X chromosome to live. Absence of X would be lethal.) Show by a checkerboard how you obtained your results.
- 13-10. In the cross of a normal female *Drosophila* and a male homozygous for the recessive transformer gene (*tra/tra*), what would be the genotypes of the parents, the F_1 , and a population of 400 F_2 flies?

Multiple Alleles and Pseudoalleles

IN THE earlier chapters we have been concerned with genes that possess only two alternative forms, or alleles. Alleles occupy the same locus in a chromosome since one of them, the mutant gene, arises from the other, the normal or wild-type gene, by mutation. Without mutation, neither the locus nor the wild-type allele would be discernible and there would be no Mendelian genetics.

For example, there is an allele that causes imperfect starch in the corn kernel. This is known as sugary, or sweet, and has the genetic symbol *su*. It is located at 71 crossover units from the end of chromosome 4. It arose by mutation from the normal starchy condition, which is labeled *Su*. Had it not been for the mutation from $Su \rightarrow su$, we would know nothing of the inheritance at this particular locus in chromosome 4.

Since we have two alleles, *Su* and *su*, the inheritance can be studied. The *su* allele, in the homozygous condition, su/su , produces a sweet corn, the kind that people prize for dinner in summer time. This *su* mutant produces a phenotype similar to the wrinkled seed mutation, one of the seven characters studied by Mendel. In both peas and corn the normal starchy condition *Su* is dominant to *su*. In the F_1 , starchy seeds are produced and in the F_2 there is a 3:1 ratio for $Su: su$. In both peas and corn only two alleles, *Su* and *su*, are known for this particular condition.

It is evident that exact laws regarding the inheritance of certain morphological characters can be determined with only two alleles at any given locus in the chromosome.

THREE OR MORE ALLELES SOMETIMES NECESSARY

In some instances it is necessary to have more than two alleles before any definite laws can be determined. A good example of this is the

self-sterility condition worked out with such precision by East and Mangelsdorf, as described in Chapter 12. You will recall that plants will not accept pollen to accomplish fertilization if the pollen is of the same genotype as either of the two self-sterility alleles in the mother plant. For example, S_1/S_2 plants will receive neither S_1 nor S_2 pollen. If only two alleles had been available, East and Mangelsdorf would not have been able to obtain any seed from the tobacco flowers. By adding another allele, S_3 , it was possible to obtain crossed seed. For example, $S_1/S_2 \times S_1/S_3$ gives two types of progeny, S_1/S_3 and S_2/S_3 , with only the S_3 pollen functioning. Hence, with three alleles it was possible to determine exact laws regarding the inheritance of self-sterility.

However, we are not limited to three alleles for self-sterility. Several more have been found in *Nicotiana*. In evening primrose, 37 different self-sterility alleles have been observed and, in red clover, more than 200. These different self-sterility alleles have undoubtedly arisen by mutation from the normal gene for no self-sterility, or possibly some alleles for self-sterility have given rise by mutation to new ones. The mechanism of mutation will be discussed more fully in Chapter 22.

OTHER MULTIPLE ALLELIC SERIES

Multiple alleles have been found in a wide variety of organisms. A number of multiple allelic series occur in the inheritance of coat color in mammals. These were discussed in Chapter 10. The *C* locus in rabbits has, besides the normal *C*, three different alleles— c^h (Himalayan), c^{ch} (chinchilla), and c^a or *c* (complete albino).

One of the characteristics of any multiple allelic series is that it is invariably at the *same* locus in the chromosome. In corn, there are several series of multiple alleles. One of these is at the *C* locus, 26 crossover units from the end of the short arm of chromosome 9. There are three alleles: C^I (an inhibitor of color), C^i (full color in presence of *A* and *R*), and *c* (colorless). Recent studies have shown that these three alleles are most likely three closely linked genes rather than true alleles. They are now commonly written $CI =$ inhibitor, Ci or $C =$ full color, and $c =$ no color.

Four main alleles for color are at the *R* locus in chromosome 10. These alleles were formerly written as R^r , colored aleurone, red color in plant; R^g , colored aleurone, green plant; r^r , colorless aleurone, red color in plants; and r^g , colorless aleurone, green plant. This is a commonly accepted method of designating multiple alleles, but the preferred way of writing them now shows them to be closely linked genes rather than true alleles. They are written as follows:

- S P* Anthocyanin color in seed, anthocyanin color in plant
- S p* Anthocyanin color in seed, no anthocyanin color in plant
- s P* No color in seed, anthocyanin color in plant
- s p* No color in seed, no anthocyanin color in plant

This terminology is similar to that used for alleles that do not belong to a multiple allelic series, but rather behave as alleles of different genes.

NO CROSSING-OVER WITHIN LOCUS

One of the characteristics of a multiple allelic series is that there is no crossing-over between alleles within a locus. For example, if an $S P/S P$ genetic stock with color in seed and color in the plant were crossed with the double recessive type $s p/s p$ and the F_1 testcrossed, there should arise but two types of progeny, as follows:

$$S P/s p \quad \times \quad s p/s p \dots\dots \left\{ \begin{array}{ll} S P/s p & \text{colored seed} \\ & \text{and plant} \\ s p/s p & \text{colorless seed} \\ & \text{and plant} \end{array} \right.$$

The proof of allelism is the lack of crossing-over within the locus. Actually a cross such as the one outlined has given a very small number of crossover types $S p/s p$ and $s P/s p$. This demonstrates that the R locus is really a compound locus and the different forms, formerly called alleles, should properly be designated *pseudoalleles*, to be discussed later in this chapter.

THE WHITE EYE LOCUS IN DROSOPHILA

The white eye in *Drosophila*, a sex-linked recessive type, was the first mutant studied by Morgan. The recessive designation is w/w . The dominant type $+$ or w^+ produces a red wild type, provided the fly has no other recessive mutant (at any locus) for eye color. To produce a red eye requires at least one dominant allele ($+$ or w^+), acting together with at least one dominant allele of all other genes affecting pigment production in the eye. No gene acts by itself, but in conjunction with all the other genes affecting the same process.

In addition to the w^+ allele producing a wild type, (red eye), and the w allele producing white, there are a number of other alleles affecting eye color. These are known by descriptive names such as ivory, pearl, buff, honey,

Table 14-1. Alleles at the White Locus in *Drosophila melanogaster*, arranged in Descending Order of Amount of Pigment Production

Allele	Symbol	Allele	Symbol
(1) Wild type (red)	$+$ or w^+	(7) Honey	w^h
(2) Coral	w^{co}	(8) Buff	w^{bf}
(3) Blood	w^{bl}	(9) Tinged	w^t
(4) Eosin	w^e	(10) Pearl	w^p
(5) Cherry	w^{ch}	(11) Ivory	w^i
(6) Apricot	w^a	(12) White	w

apricot, cherry, blood, and coral. They are all at the same locus (location) 1.5 units from the end of chromosome 1. Table 14-1 shows the colors, ranging from the white through the various shades to the wild-type red, and the commonly accepted way of writing the genic constitution of alleles at a single locus. These alleles have all arisen by mutation and they can mutate to one another or to the dominant wild type w^+ . More than 15 alleles have been found at the white locus. Possibly more would be known if we were able to distinguish a finer gradation of colors.

DOMINANCE WITHIN AN ALLELIC SERIES

Table 14-1 lists the different alleles at the white locus in descending order of pigment production. The wild-type allele (w^+) is dominant to all of the others and there is a lack of dominance for alleles other than the wild (w^+). For example, the genotypes w^+/w^{co} , w^+/w^e , w^+/w^a , w^+/w^h , w^+/w^i and w^+/w all have red eyes, characteristic of wild flies. However, for the intermediate alleles such as a genotype w^a/w , the heterozygote is not as dark as w^a/w^a but rather intermediate between w^a/w^a and w/w .

This is understandable if we consider how the genes act to produce their end products. The best assumption is that they produce enzymes that catalyze the formation of some product, in this case pigment in the eye. Apparently one allele of w^+ produces sufficient enzyme to develop full color. Such is not true for the intermediate alleles, each of which is limited in the amount of enzyme it produces. In these cases the effect is cumulative, two alleles doing a better job than one. In the case of the white allele (w), no enzyme production results in no pigment, consequently a white eye.

COAT COLOR IN MAMMALS

Several different multiple allelic series in mammals have been mentioned. The dominance relation will now be discussed. In the case of the C series in rabbits with four alleles, C , c^{ch} , c^h , and c (or c^a), the dominance of C is complete over all three other alleles. C/c^{ch} , C/c^h , and C/c^a are wild type, just as surely as C/C . The order of dominance is C , c^{ch} , c^h and c^a . The allele c^{ch} is not dominant to c^h or c^a , but animals that are c^{ch}/c^h or c^{ch}/c^a are light gray rather than chinchilla. Rabbits that are c^h/c^h or c^h/c^a are Himalayan. In this case the allele c^h is dominant to c^a (the albino allele). The allele c^a (also written c) is the lowest order of the series producing no pigment.

In dogs there are three distinct alleles at the A locus.

A^s causes dark pigment to be distributed throughout the whole body. Good examples are Labrador and Chesapeake Bay Retrievers.

a^y greatly restricts the dark pigment, giving rise to the sable coat or tan-colored dogs, as found in Collies.

a^t produces bicolor varieties, black and tan, liver and tan—colors described as *tan points*. Good examples are Welsh Terriers, and Doberman Pinschers.

The order of dominance is $A^* \rightarrow a'' \rightarrow a^t$.

Certain similarities of the multiple allelic series are listed below:

1. Multiple alleles are always at the same location (locus) in the chromosome.
2. There is no crossing-over within a multiple allelic series. When two alleles are involved in a cross, the same two alleles are recovered in the F_2 or testcross progeny.
3. Multiple alleles always affect a similar character. For example, all of the alleles at the white locus affect eye color and not body color, wing size, and venation or bristle number. This indicates a similar origin of the different alleles.
4. The wild-type allele is nearly always dominant. The other alleles in the series may show dominance, or there may be intermediate phenotypic effects when two different alleles are brought together in the same genotype.
5. The last characteristic of multiple alleles is that, when any two are crossed, the phenotype is of a mutant character and not the wild type. Thus, if a w^a/w^a ♀ (apricot) fly is crossed by a w (white male), no wild-type flies appear in the F_1 , but rather a mutant type intermediate between apricot and white. This is in contrast to crossing different genetic stocks, such as vermilion and cherry, which are similar phenotypically. Since the mutants vermilion and cherry each have the dominant allele of the other, they are said to complement each other. This test of complementary action can be used to distinguish alleles from non-alleles. Stocks with non-allelic genes produce normal types when crossed. Alleles of the same gene when crossed produce a mutant phenotype.

These five criteria are applied to alleles suspected of being alleles of a single wild-type gene. If two suspected alleles fail any one of these five tests, they are probably not alleles, but pseudoalleles.

One of the first demonstrations of a pseudoallelic condition was that of the Star-asteroid case analyzed by E. B. Lewis in 1951. He found a recessive mutation in *Drosophila*, producing a small rough eye when homozygous. It was at locus 1.3 in the second chromosome. This was the identical location of Star, a dominant mutation also affecting the morphology of the eye. The eye was rough and had a slight gleam—hence the name Star, with the gene symbol S .

By two of our criteria, same location in the chromosome and having a similar function, these would be classed as alleles. A third, lack of crossing-over, also indicated they were alleles. Crosses of Star and the Star-recessive

produced, in a reasonable number of flies, only Star and Star-recessive. However, when the progenies included a great many flies, a low proportion, about one in five thousand, of recombinations occurred. The Star-recessive was then labeled "asteroid" with the gene symbol *ast*. Thus the two supposed alleles were demonstrated to be pseudoalleles.

It was possible to produce two different kinds of heterozygotes as follows:

$$(1) \ S^+ ast/S ast^+ \quad \text{and} \quad (2) \ S ast/S^+ ast^+$$

It was found that these two types of heterozygotes, with the same alleles, but in different combination, were different phenotypically.

The first heterozygote had an eye much reduced in size, while the eye of the second was normal size (Fig. 14-1).



Courtesy of E. B. Lewis, Cold Spring Harbor Symposium, 1951

FIG. 14-1 Position effect in *Drosophila*. Both flies are heterozygous for star and asteroid. The fly at left has mutant allele on each chromosome $S^+ ast/S ast^+$, the *trans* arrangement. The one at right has two mutant alleles on one chromosome, with two normal alleles on the other, the *cis* arrangement.

This is an excellent example of *position effect*, where the phenotype is determined not by the total alleles present in the organism (the genotype), but by the position in the chromosomes of the different alleles. If the fly has two normal alleles in the same chromosome, while the two mutant alleles are in the homologous chromosome, a fully developed eye results.

When the two mutant alleles are located in one chromosome and the two "normal" alleles in the other, it is known as the *cis* arrangement (from the Latin *cis* meaning "on this side"). The alternative form is known as the *trans* arrangement. Apparently the two normal alleles must be in the same strand of chromosome to function normally.

POSITION EFFECT COMMON FOR PSEUDOALLELES

The pseudoallelic condition just cited for Star and asteroid has been found in other multiple-allelic series of a wide variety of organisms in ad-

dition to *Drosophila*, including corn, cotton, *Aspergillus*, and *Neurospora*, and in bacteria and viruses. In fact, in any organism susceptible of precise analysis, pseudoalleles have turned out to be the rule rather than the exception. Exact analysis is scarcely possible in some larger animals such as mice, rabbits, dogs, and horses because of the extremely large number that must be studied to detect pseudoalleles. If it were possible to analyze carefully any set of multiple alleles, it would possibly be found that they were really pseudoalleles. The classical example of multiple alleles at the white locus in *Drosophila*, mentioned earlier in this chapter, has been shown to consist of at least four parts. Crossing-over between apricot and eosin occurs at a rate of about 10^{-4} , or once in 10,000 meioses.

This careful analysis of different sets of pseudoalleles suggests perhaps a common origin for all multiple alleles at a given locus, whether they be true multiple alleles or perhaps, as seems more likely, pseudoalleles. All have similar physiological and chemical functions, and they all may have been derived from the same original gene by a process of duplication through a mistake in crossing-over.

The origin of the Bar mutation in *Drosophila* is now well understood. It represents the duplication of a small piece of chromosome at locus 57 in chromosome 1.

POSITION EFFECT AT THE BAR LOCUS

The dominant gene Bar in *Drosophila* was analyzed genetically by Sturtevant and Morgan (1923) and was shown to consist of the duplication of a segment of chromosome 1. They found that *B/B* females did not invariably give *B/B* flies, but about one in 1600 offspring had a normal eye.

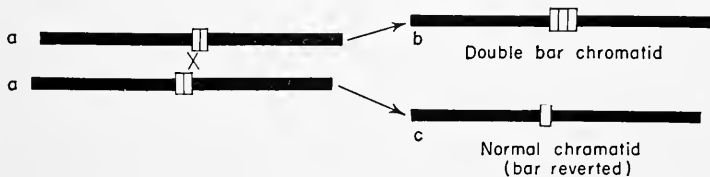


FIG. 14-2 Diagram showing unequal crossing-over in the duplicated Bar section of chromosome 1 in *Drosophila*, giving one strand with three segments (double Bar), and one with a single Bar segment (wild type).

In the same proportion, flies were produced that were called double bar because the facet number was reduced much more than Bar.

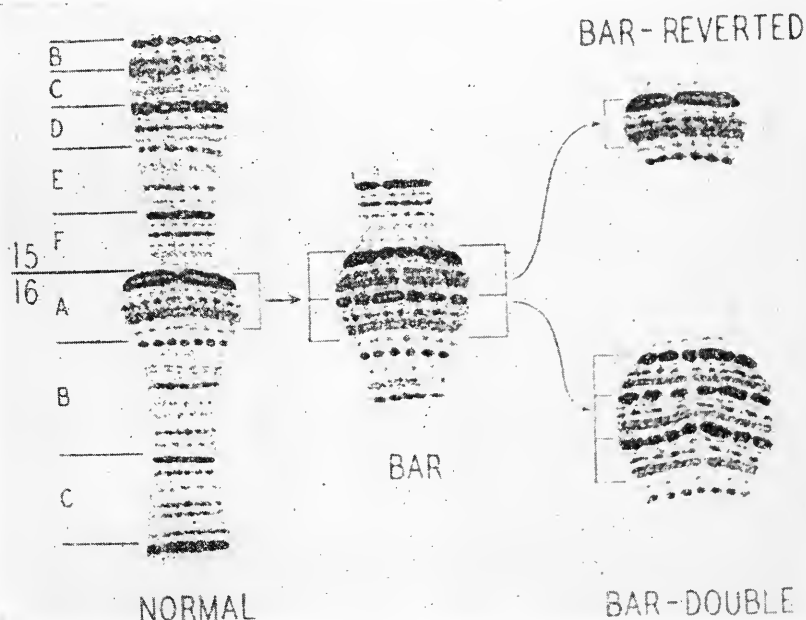
The origin of these two types could be explained by the assumption that Bar is really a duplication of a small chromosomal segment and that unequal crossing-over took place between these two segments (Fig. 14-2).

After salivary gland chromosomes in *Drosophila* became available for

analysis, C. B. Bridges (1936) studied the Bar region cytologically. He was able to see the exact region that had been duplicated (Fig. 14-3).

Since the Bar "gene" is really a duplication of a short segment of the X chromosome, the homozygous B/B female fly has a duplicated segment in each chromosome (Fig. 14-2). This makes a total of four segments.

Following unequal crossing-over within this segment of the X chromosome, there results one chromatid with three segments, the other with one (Fig. 14-2). The one with three segments is called double Bar, while the chromatid



From Bridges, and Science, 1936

FIG. 14-3 Bar region of X chromosome in *Drosophila*. Bar represents a duplication of five bands. This region reverts occasionally to wild type and triple Bar.

with one segment is the normal wild type, or Bar-reverted chromatid. Flies possessing a double Bar chromosome and a wild-type chromosome show a more extreme manifestation of the Bar phenotype than does a fly with equal distribution of the Bar segment in both chromosomes. Both types of flies possess four of the Bar segments, but in different distribution between the two chromosomes. As a result they have eyes phenotypically different. This is another good example of position effect. Organisms with the same genotype may have phenotypes that are markedly different, depending upon the distribution of the genetic elements. In the case of the Bar-eyed *Drosophila*, the genetic element is a short chromosomal segment, rather than a gene.

ORIGIN OF NEW GENES

The Bar case just cited is one in which the origin of a hereditary determiner is well understood. Bar was originally termed a dominant gene, until its true value was understood.

Possibly a wide variety of alleles, or pseudoalleles, at any given locus may have arisen similarly by duplication, but with infinitely smaller chromosomal segments involved. The fact that, in any multiple allelic (or pseudoallelic) series, the different alleles affect a similar process is an argument for a common origin. A new allele might be expected to affect a similar process.

BLOOD GROUPS IN HUMAN BEINGS

It has been demonstrated that the inheritance of different blood groups in man is determined by a number of multiple allelic series. Possibly these different systems may be really pseudoallelic systems with rare crossing-over. The human animal is not as well adapted for establishing this point as some of the lower forms.

It has been known since the pioneer work of Landsteiner (1900) that, when red blood cells of one person were mixed with serum of another person, in some cases the cells would be clumped or agglutinated. He found that people could be divided into four groups, according to which *antibodies* (*agglutinins*) were present in the serum and which *antigens* (*agglutinogens*) were present on the red blood cells.

These antigens (mostly proteins) are but a few of those found on the red blood cells of man. These antigens are of extreme importance when blood transfusions are made. We are primarily concerned with how they are inherited, but a brief explanation of how they operate seems desirable.

The four groups discovered by Landsteiner have different agglutinogens on their blood cells, and each group possesses a characteristic agglutinin body in the blood serum. There are two main agglutinogens, A and B, for which two different alleles are responsible. A third allele causes *no* agglutinin to be produced on the blood cell. This condition is recessive to either A or B, which are codominant. These three genetic types are all alleles of but a single locus in one of the 22 autosomal chromosome pairs of man.

The different blood groups are known as A, B, AB, and O. Individuals in the A group have agglutinin A on the red blood cells, B agglutinogens B; AB has both agglutinogens A and B, while group O has neither.

The genotypes for the different blood groups have been designated in various ways. Some prefer the system using a superscript of the capital letter L for Landsteiner, the discoverer. According to this terminology, group A would be L^A/L^A (if homozygous); group B, L^B/L^B (if homozygous); group AB

would be L^A/L^B while group O would be designated by l/l , showing the recessive nature of the allele.

Other workers use a similar designation but with superscripts of the letter I for *isoagglutination*, the technical term for the agglutination or clumping of the red blood cells by an agglutinin. The prefix *iso* [from the Greek *isos*, meaning equal] indicates that the agglutination is caused by sera from the same species, man.

In this text we shall use for gene symbols the same letters designating the blood groups, *A* for agglutininogen A, *B* for B, *A/B* for AB, and *O* for group O, bearing in mind that A and B are codominant, while O is recessive to both.

Each blood group has a characteristic antibody or agglutinin in the serum. These agglutinins are commonly known as anti-A and anti-B. If red blood cells of type A are placed in a serum with the agglutinin anti-A, the red blood cells will be clumped or agglutinated. In a similar manner B blood cells are clumped by a serum with anti-B. Such reactions have serious consequences in blood transfusions. An incompatible relationship would result in clumping of the donor's red blood cells when received, and most likely in the death of the patient. In making blood transfusions it is imperative that the blood group of both the donor and recipient be known. To be absolutely safe, transfusions should be made only between persons belonging to the *same* group. A word about "universal donors" and "universal recipients" will be added following an explanation of the antigens or agglutinins in the serum.

Blood group A has in its serum the agglutinin Anti-B and should any B blood be introduced, the red blood cells of B will be clumped or agglutinated. Likewise blood group B has anti-A and any A blood cells introduced into B serum would be agglutinated. It is evident that a person in blood group A cannot have any anti-A agglutinin in his serum, or his own red cells would be agglutinated. Likewise individuals in group B have no anti-B agglutinin in their serum. Those in group AB can have neither antigens anti-A or anti-B, and are sometimes called universal recipients, because their serum will not coagulate any red blood cells introduced.

Persons in blood group O, while possessing no agglutinogens on their red blood cells, have agglutinins anti-A and anti-B in their serum and can receive only type O blood in transfusion. Since they have neither agglutinogens A or B, their blood is often used for transfusions of patients A, B, or AB. Group O is sometimes called a universal donor.

The above relationships concern the red blood cells introduced into the serum of the recipient. This is the reaction of most importance. However, in any whole blood transfusion some of the serum of the donor is introduced, as well as red blood cells. Apparently most of the agglutinins in the introduced serum are quickly adsorbed to the tissues of the recipient, and the serum is quickly diluted by the serum of the recipient, so that serious agglutination may not take place.

A thorough discussion of this situation is found in Curt Stern's *Principles*

of *Human Genetics* (1960). He states, "Since the effect of the donor's antibodies, if present, is not wholly negligible, the terms universal donor and recipient are not fully correct and the use of any donor different in blood group from the recipient should be discouraged."

Table 14-2 is a reproduction of Table 21 of Stern. This shows the agglutination relationships when whole blood is used in transfusions.

Table 14-2. Possible Effects of Transfusions of Whole Blood^a

Donor \ Recipient				
	O	A	B	AB
O	—	+	+	+
A	+	—	+	+
B	+	+	—	+
AB	+	+	+	—

Note: + = heavy agglutination of donor's cells
 + = light agglutination of recipient's cells
 — = no agglutination

^a Courtesy Curt Stern and the Freeman Co.

Table 14-3 lists the different blood groups, with the genotypes of each, and also the agglutinogens and agglutinins possessed by each group.

Table 14-3. Characteristics of ABO Blood Groups in Man

Blood Group	Genotype	Blood Contains		Red Blood Cells— Reaction with Antibodies	
		Cellular Agglutinin	Serum Agglutinin	Anti-A	Anti-B
A	A/A A/O	A	Anti-B	+	—
B	B/B B/O	B	Anti-A	—	+
AB	A/B	A B	None	+	+
O	O/O	None	Anti-A Anti-B	—	—

DETERMINATION OF BLOOD GROUP

In determining the blood group of a person, a small sample of his red blood cells, or of whole blood, is mixed with different sera. One frac-

tion is mixed with anti-A agglutinin, and another with serum containing anti-B agglutinin. If agglutination occurs with anti-A agglutinin, it is obvious that the blood group of the donor of the cells is A. If agglutination occurs with anti-B agglutinin, the blood group is B. If agglutination occurs in both Anti-A and Anti-B sera, the donor's blood group is AB, but if in neither his group is O.

The different sera for use in the test can be obtained from known blood groups, serum with anti-A from B individuals, and serum for anti-B from persons of group A.

GENETICS OF ABO BLOOD GROUPS

As a result of the study of thousands of families representing all possible matings between and within the different blood groups, the inheritance of the A, B, and O alleles has been determined.

The accumulated data support the hypothesis that there are three alleles concerned with the determination of the blood group of any given person. Each individual can have any two of these alleles, which are located in one of the 22 autosomal chromosome pairs. Table 14-4 shows the genotypes of different parental combinations, with the possible genotypes of the progeny, and also genotypes not possible from a given combination. Data of this kind have been used in court cases of disputed parentage. The last column of the table tells why one can say that a certain genotype in a child is *not* possible

Table 14-4. Blood Groups and Genotypes of Parents and Offspring
(also shown is the blood group not possible in offspring.)

Parents		Offspring		Impossible Group
Groups	Genotype	Groups	Genotype	
O × O	O/O × O/O	O	O/O	A, B, AB
O × A	O/O × A/A	A	A/O	
O × A	O/O × A/O	O, A	O/O, A/O	
O × B	O/O × B/B	B	B/O	O, A, AB
O × B	O/O × B/O	O, B	O/O, B/O	
O × AB	O/O × A/B	A, B	A/O, B/O	
A × A	A/A × A/A	A	A/A	O, B, AB
A × A	A/O × A/O	A, O	A/A, A/O, O/O	
A × A	A/A × A/O	A	A/A, A/O	
A × B	A/A × B/B	AB	A/B	O, A, B
A × B	A/A × B/O	A, AB	A/O, A/B	
A × B	A/O × B/B	B, AB	B/O, A/B	
A × B	A/O × B/O	A, B, AB, O	A/O, B/O, A/B, O/O	
AB × AB	A/B × A/B	A, B, AB	A/A, A/B, B/B	O

from the parental genotypes given. On the other hand, one cannot always say definitely that a specific child was the result of a given combination.

THE MN SERIES OF BLOOD GROUPS

In the ABO series discussed, the agglutinogens are present on the red blood cells and the agglutinins in the serum. Consequently, one must be careful to match blood types in making transfusions, so that the introduced blood cells are not agglutinated by an antagonistic serum.

In the case of the MN series, the human blood serum contains no antibodies that would agglutinate any blood cells. If human blood is injected into rabbits, however, the rabbit develops antibodies in its serum that are antagonistic to the different antigens introduced with the blood cells. Landsteiner and Levine in 1927 found that the human population could be divided into three different groups that reacted differently with the antibodies developed in the rabbit (Table 14-5). These reactions occur only after injecting human blood into the animal, and are of no consequence in blood transfusions.

According to Crow, the MN locus has been found to be more complex. Readers are referred to Crow for a discussion of the other pair of genes *S* and *s*.

Table 14-5. MN Series of Blood Groups Showing Reactions of Different Genotypes with Antibodies Developed in Rabbit Serum

Blood Group	Genotype	Red Blood Cell Reaction with Antibodies		Blood Contains		Frequency in U.S. White Population ^a
		Anti-M	Anti-N	Cellular Antigens	Serum Antibodies	
M	M/M	+	—	M	None	26%
MN	M/N	+	+	M, N	None	53%
NN	N/N	—	+	N	None	21%

^a Data from Crow's *Genetics Notes* (1960). Courtesy of James F. Crow.

THE RHESUS RH REACTION

In 1940 Landsteiner and Levine reported yet another set of blood antigens. Following the injection of the blood of Rhesus monkeys into rabbits and testing for all previously known antigens, it was found that the blood of about 85% of all human beings tested still was agglutinated by the antigen in the treated rabbit serum. These human beings contained a blood antigen in common with the Rhesus monkey, and the symbol Rh+ was used to show the similarity. About 15% of the human population showed no reaction (Rh—).

R. A. Fisher has proposed a system of nomenclature whereby the antigen in the Rh+ individuals is labeled D. Rh- people have the genotype *d/d*. The reactions of the Rh series are shown in Table 14-6 (after Crow). Readers are referred to Crow for a fuller discussion of the Rh system. Recent studies have shown that the Rh system is much more complex than was once believed.

Table 14-6. Description of Rh Blood Group in Man

Blood Group	Genotype	Red Blood Cell Reaction with Anti-D Antibody	Blood Contains		Frequency in U.S. White Population
			Cellular Antigens	Serum Antibodies	
Rh+	<i>D/D</i> , <i>D/d</i>	+	D	None	85%
Rh-	<i>d/d</i>	-	None	None ^a	15%

^a Capable of producing anti-D antibodies after exposure to cells with D antigen.

ERYTHROBLASTOSIS FETALIS AND THE RH FACTOR

As shown in Table 14-6, Rh- (*d/d*) individuals possess no antibodies for the D antigen, but may develop such antibodies after repeated exposure to D antigens. This may occur after repeated transfusions of Rh- (*d/d*) persons with blood from Rh+ persons. Apparently the first transfusions are successful, but antibodies are built up in the serum of the Rh- person and these antibodies may attack the D antigens on Rh+ cells introduced later. In this case the reaction is one of agglutination of the red cells of the Rh+ (D) person.

Another way in which antibodies may be developed is in the pregnancy of an Rh- woman carrying a fetus that is Rh-/Rh+. The father is Rh+, either *D/D* or *D/d*. Apparently little trouble is experienced in the first and second pregnancies. By the time of the third, however, the mother is likely to develop anti-D antibodies that are passed across the placental membrane and that react with the developing fetal blood to cause its destruction.

Medical scientists have learned how to save such infants by immediately withdrawing most of the blood at birth and replacing it with blood containing no anti-D antibodies.

This type of Rh- with Rh+ incompatibility gives serious consequences in only about one out of 200 to 300 human births in North America. The first and second children are usually not affected, but trouble may be expected with the third and later pregnancies.

In making transfusions, it is a wise precaution not to give an Rh- person a transfusion with blood that is Rh+, as antibodies will be developed sooner or later. Since only 15% of the population is Rh-, it may always

be difficult to find an Rh— donor for a Rh— recipient. Repeated transfusions are certainly to be avoided.

CONCLUDING REMARKS

This chapter is concerned with the inheritance of genes that possess more than two alternative forms, allelomorphs, commonly known as alleles. Multiple alleles have several things in common:

1. They are always at the same locus in the chromosome.
2. There is no crossing-over between multiple alleles. This qualification has had to be modified, as many multiple alleles have been shown to be pseudoalleles. Possibly many more, perhaps all, multiple alleles would be found to be pseudoalleles if our techniques were sufficiently precise.
3. They always affect a similar character, i.e., eye color, eye shape, seed and plant coloring, etc.
4. The wild-type allele is usually dominant to all others at the same locus. The other alleles may show dominance or they may have an intermediate phenotypic expression.
5. The last characteristic of multiple alleles is that when any two are crossed, the phenotype is of a mutant character and not the wild type. This is sometimes called *lack of complementarity* of multiple alleles.

The different multiple alleles, or pseudoalleles, possibly have a common origin, having mutated from a common gene. Some of the alleles may have given rise to others by mutation. The mechanism of the production of new genes has been shown.

The inheritance of different blood groups in human beings is a good example of the operation of multiple allelic series. The genetics of three different series, the ABO, the MN and the Rh+ Rh— series, are presented. Problems in blood transfusions are described, with procedures to be avoided.

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PROBLEMS

- 14-1. Define or describe the following terms.

ABO blood groups (human)	multiple alleles
agglutinins	multiple alleles at <i>A</i> locus (mammals)
agglutininogen	multiple alleles at <i>w</i> locus (<i>Drosophila</i>)
antibody	MN blood groups (human)
anthocyanin	position effect
antigen	pseudoalleles
chinchilla rabbit	pseudoalleles, <i>cis</i> arrangement
double Bar (<i>Drosophila</i>)	pseudoalleles, <i>trans</i> arrangement
erythroblastosis fetalis	Rh+ and RH—
Himalayan rabbit	self-sterility alleles
inhibitor of aleurone color	universal donor

- 14-2. Identify the following scientists, giving a major contribution with an approximate date:

Bridges, C. B.	Landsteiner, K.
Crow, James	Levine, P.
East, E. M.	Lewis, E. B.
Fisher, R. A.	Mangelsdorf, A. J.
Green, M. M.	Stern, Curt

- 14-3. In the cross of *Drosophila* Star (*S*) and asteroid (*ast*), the F_1 has an eye much reduced in size. In the F_2 the parental types are recovered in most cases, but occasionally (one in 5000) a fly with normal eyes appears. Show how this may be accounted for by crossing-over within the locus. What other type of crossover would be expected? Why would it most likely not be observed? Label properly the *cis* and *trans* heterozygotes.
- 14-4. In *Drosophila* there are several alleles at the white locus in the sex chromosome. Beginning with the normal allele w^+ with a red eye, the amount of pigment present diminishes until the white (w) is reached. the w^+ allele is dominant to all others. When the intermediate alleles are crossed, they show a cumulative effect with the F_1 intermediate to the two parents. One of these alleles is apricot eye which is designated w^a .
- Make a cross between an apricot female and a white male. Give the genotypes and phenotypes of the F_1 males and females.
 - Give the same information for the reciprocal hybrid.
- 14-5. The genes for apricot and white (Problem 14-4), once thought to be allelic, have been shown to be pseudoalleles, with rare crossing-over. One of the following types has a normal wild-type eye, the other light apricot. They are now labeled as pseudoalleles rather than alleles. The two types are $aw/++$ and $a+/+w$.
- Label properly the phenotypes of the two genotypes. Give a possible explanation why one should be wild, the other light apricot.
- 14-6. In rabbits the following combinations of alleles at the *C* locus give the phenotypes named:

Genotype			Phenotype
C/C			wild type
C/c ^{ch}	C/c ^h	C/c ^a	wild type
c ^{ch} /c ^{ch}			chinchilla
c ^{ch} /c ^h	c ^{ch} /c ^a		light gray
c ^h /c ^h			Himalayan
c ^h /c ^a			Himalayan
c ^a /c ^a			albino

Give the ratios to be expected in the following crosses:

- $C/c^a \times C/c^{ch}$
- $c^{ch}/c^a \times c^{ch}/c^a$
- $C/c^h \times c^{ch}/c^h$
- $c^a/c^a \times c^{ch}/c^h$
- $c^{ch}/c^h \times c^h/c^a$

- 14-7. At the R locus in chromosome 10 of maize are four alleles for seed and plant color. (Actually they are most likely pseudoalleles with extremely rare crossings-over, and this may be ignored in the solution of your problem.) They have been designated by two methods as follows:

- | | |
|-------------------------------------|---------------|
| (a) Colored aleurone, red plant | R^r or SP |
| (b) Colored aleurone, green plant | R^g or Sp |
| (c) Colorless aleurone, red plant | r^r or sP |
| (d) Colorless aleurone, green plant | r^g or sp |

A cross was made between (a) and (d). Show complete genotype of parents and F_1 . What is the phenotype of the F_1 seed and the F_1 plant? In 1000 F_2 (seeds and plants) how many of the different classes will there be? (The F_1 seeds can be classified on the ear while it will be necessary to grow seedlings to classify plant color.)

- 14-8. In human beings three different series of multiple alleles are concerned with blood groups, ABO, MN, and Rh. Which of the following children are possible from the parents given?

Mother			Father			Child		
(a) O	N		O	M		A	N	
(b) A	MN	Rh +	B	N	Rh +	O	N	Rh -
(c) AB	M	Rh -	B	N	Rh +	A	MN	Rh -
(d) B	M	Rh -	A	M	Rh -	O	MN	Rh -
(e) O	MN	Rh +	B	N	Rh +	O	N	Rh -

- 14-9. The Rh+/Rh- system was further subdivided into a series of multiple or pseudoalleles. Rh- is labeled *cde* and Rh- persons are *cde/cde*. Rh+ persons have different combinations of CDE or combinations of large and small letters. The three letters go together as a group. The following problem uses this system of nomenclature for Rh+ and Rh- persons. The Rh designation is shown in parentheses.

In a case of disputed parentage, which of the two men could not have been the father? Give all of the reasons you can think of why not.

Woman	A	M	<i>cde/cde</i>	(Rh - / Rh -)
Child	O	MN	<i>cde/cde</i>	(Rh - / Rh -)
Man 1	AB	M	<i>CDE/CDe</i>	(Rh + / Rh +)
Man 2	B	N	<i>cDE/cde</i>	(Rh + / Rh -)

14-10. In horses there are most likely four alleles at the A locus. (See Table 10-3.)

A^+ (with B)	wild-type Prejvalski horse
A (with B)	bay—black mane and tail
a^+ (with B)	brown—almost black with lighter areas
a (with B)	black—solid color

If you bred several bay mares whose sire was a brown, to a brown stallion whose sire was a black, what type of progeny would result and in what proportion?

Assume you obtained at least one male of each possible genotype from these matings. If these stallions were each used on a herd of black mares, what types of progeny would be expected?

Cytoplasmic Inheritance and Influence

IN PRECEDING CHAPTERS we have been concerned with the inheritance of characters whose genes are located in the chromosomes. Much has been learned regarding the way these genes are passed on from parent to offspring. They are divided and distributed with great precision at the reduction division, so that the gametes formed have a random assortment of genes of the parents. This distribution was postulated by Mendel, and cytological researches have shown that the segregation of the chromosomes was the plausible mechanism for the distribution of the genes.

Because the genes are an integral part of the chromosome does not mean that the rest of the cell, the cytoplasm, is nonessential to the functioning and expression of hereditary characters. Actually, there is much more cytoplasm than nuclear material, even though the cytoplasm is not divided and passed on to future cells with the precision shown in the distribution of the nuclear material. The parts of the cytoplasm are distributed more or less at random when a mother cell divides to form two daughter cells. The cytoplasm is the medium in which the nucleus exists. The genes reduplicate themselves in the nucleus and initiate the action that gives rise to the hereditary characters. Actually, the material from which a gene reduplicates itself is transported in the cytoplasm, and some of the cytoplasmic material is used in this reduplication process.

The cytoplasm is rich in ribonucleic acid (RNA), some of which occurs also in the nucleus. One of the theories regarding the role of deoxyribonucleic acid (DNA), in carrying genetic information, suggests that "the information in genetic DNA is first translated in RNA and that it is the RNA that serves as templates for protein synthesis. This could be called the hypothesis of indirect or two-step translation" (Beadle, 1957). Beadle states further, "There are several reasons for preferring the second hypothesis. One of them is that

plant and animal viruses are known that are made up of RNA and protein, with no DNA at all. In these, primary genetic information must be carried in the form of RNA."

Our understanding of how the genetic specifications in the DNA of the chromosomes are translated into the formation of specific characters has increased rapidly in the last decade or two. As Beadle postulates, the RNA, found both in the cytoplasm and the nucleus, most likely plays an active part in the translation of these directions into results.

It is known that most hereditary characters have their determiners in the chromosomes, inside the nucleus. There are cases, however, where the cytoplasm seems to determine the inheritance directly, and not by the translation of genetic specifications carried in the chromosomes.

PLASTID INHERITANCE IN PLANTS

There are many cases in plants where the inheritance of abnormal plastids is determined by specific genes in the chromosomes. Witness, for example, in corn more than a score of genes for *virescent* seedlings with greater reduced amounts of chlorophyll, also a comparable number of genetic types of albino seedlings devoid of chlorophyll and generally of chloroplasts. There are definite cases of chloroplast abnormalities conditioned by single, sometimes duplicate, gene differences from the normal green. In addition instances are known where plastid inheritance is passed on directly from cytoplasm to cytoplasm with no gene in the chromosome directing the operation, or "calling the signals." In these cases the inheritance is through the maternal side only, since the egg contains a small amount of cytoplasm, while the sperm from the pollen is practically devoid of it.

One of the cases most carefully analyzed is that of the four-o'clock, *Mirabilis jalapa*, whose plants have areas of pale green and white in otherwise normal green leaves. This variegated condition may extend to a considerable portion of the plant. In such specimens there may be branches that are wholly green, wholly light colored, or showing the variegation of light, normal green, and mixed tissues.

Within a normal green sector, the chloroplasts are normal and give rise to normal chloroplasts. In the pale sectors, pale chloroplasts are transmitted, and the variegated sectors seem to have a mixture of pale and normal green chloroplasts.

By pollinating flowers produced on branches of the three different types with pollen from the three different types, it was possible to determine the relative importance of the pollen versus the egg in transmitting the variegated condition. Such pollinations have demonstrated that the *egg alone* is responsible for transmitting the normal or pale chloroplasts. The pollen has no effect. This is shown diagrammatically in Fig. 15-1.

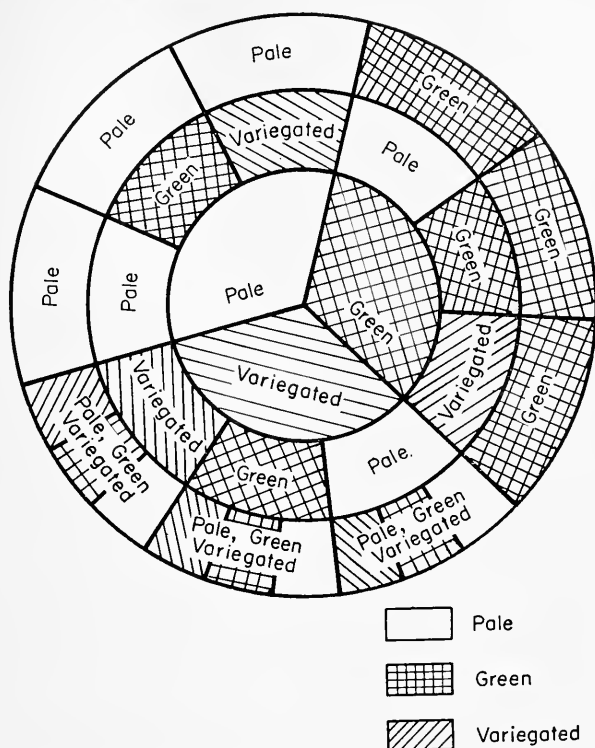


FIG. 15-1 Diagram of plastid inheritance in four-o'clocks. The central circle represents the type of branch that produces the flower pollinated. The intermediate circle represents the branch producing the flower used for pollen. The outer circle shows progeny, which is determined solely by the inner circle, or the branch producing the flower pollinated.

Similar behavior has been found in corn, sorghum, beans, and other plants. Although the cases are few compared to the many conditioned by genes in the chromosomes, there is no doubt that in rare instances inheritance can be determined by particles located in the cytoplasm. These particles are now spoken of as *plasmagenes*, in contrast to the genes in the chromosomes, sometimes called *chromogenes* to distinguish them from plasmagenes.

TEAMWORK BETWEEN GENES AND PLASMAGENES

In some instances inheritance seems to be determined by a combination of genes and plasmagenes. Two examples will be given. One is from the classic work of Sonneborn and his colleagues in determining the inheritance in *Paramecium*. The other comes from Rhoades' careful analysis of the perplexing inheritance of *iojap* in corn, which will be discussed first.

IOJAP INHERITANCE IN CORN

Iojap is a striking condition in corn, discovered by Merle T. Jenkins in Iowa and first reported in 1924 (Fig. 15-2). It was named iojap because it was discovered in Iowa and it was similar to "japonica," a type of striping due to a recessive gene. The plants are quite decorative with their sharply contrasted stripes of green and white. The gene responsible for japonica is in chromosome 8. The inheritance is entirely genic, with reciprocal hybrids showing the same type of inheritance. The gene *j* is recessive to *J* so that the F_1 hybrids J/j (made either way) are green, and good 3:1 ratios are obtained in the F_2 , a typical monogenic segregation.



Courtesy of M. T. Jenkins;
and *Journal of Heredity*

FIG. 15-2 Iojap striping in corn, resulting from a cooperation between a gene in chromosome 7 and the cytoplasm.

There is a gene partially responsible for iojap. It is located in chromosome 7. Its inheritance presents a marked contrast to that of japonica, as has been shown by Rhoades. Unlike japonica, which is phenotypically striped only when homozygous recessive, j/j , the iojap striping appears when the mother plant was either ij/ij or Ij/ij , with a striped phenotype. Reciprocal hybrids between green and iojap are decidedly different (Table 15-1).

Table 15-1. Unusual Genetic Transmission of Iojap Striping in Corn

li/li ♀ (green) \times ij/ij ♂ (iojap)..... li/ij (all green)

ij/ij ♀ (striped) \times li/li ♂ (green)..... $\begin{cases} (1) li/ij \text{ (green)} \\ (2) li/ij \text{ (striped)} \\ (3) li/ij \text{ (white: lethal)} \end{cases}$

Progeny of type 2 (above) pollinated by li/li (green)

2. li/ij (striped) \times li/li (green) = $\begin{cases} (1) li/li \text{ (green)} \\ (2) li/li \text{ (striped)} \\ (3) li/li \text{ (white: lethal)} \\ (4) li/ij \text{ (green)} \\ (5) li/ij \text{ (striped)} \\ (6) li/ij \text{ (white: lethal)} \end{cases}$

In the case of iojap the striped condition is apparently inherited through the maternal side only. It seems there are two kinds of plastids, one normal green and the other abnormal, or iojap. These plastids are distributed at

random. Should an egg receive only normal plastids, the resultant seed produced by fertilization with any pollen grain would be green. If, perchance, all of the plastids that went into one egg were abnormal, a white seedling would be the result. If, however, an egg received a mixture of normal and abnormal plastids, the plant would be striped.

Apparently the *ij* gene in chromosome 7 initiates the production of abnormal plastids which can be transmitted through the cytoplasm to eggs of any genetic constitution for the *ij* locus, either *Ij/Ij*, *Ij/ij*, or *ij/ij*. Once established, this character of the cytoplasm may become continuous and influence the phenotype.

What about the plastids of plants homozygous for *ij/ij*? They cannot all be of the abnormal type, else they would produce all white seedlings, which would be the end of the story. Apparently, some normal plastids occur in *ij/ij* plants, giving rise to a striped condition. Possibly the abnormal plastids mutate occasionally to normal ones. Somehow, there is an interaction between the genes in a chromosome (number 7 in this instance) and the cytoplasm. This is somewhat analogous to the situation in *Paramecium*, analyzed so carefully by Sonneborn and his associates.

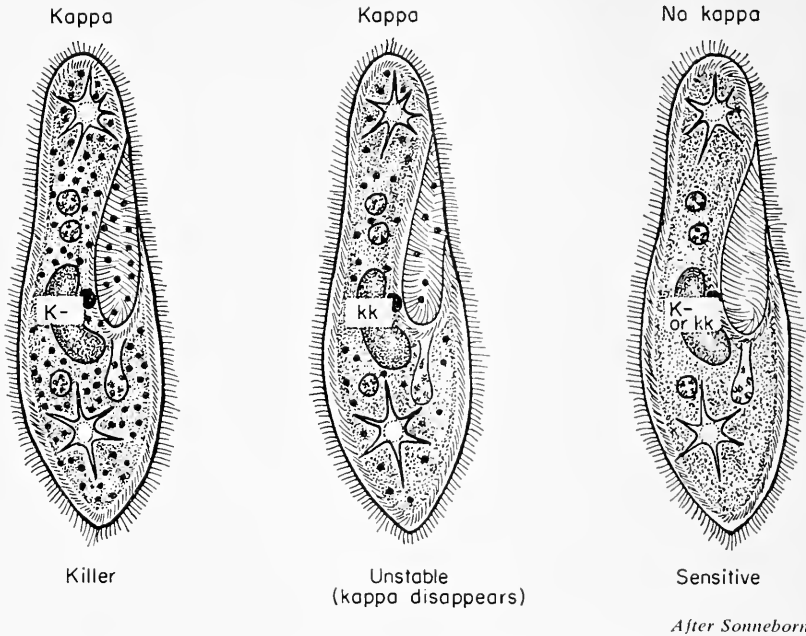
INTERACTION BETWEEN GENES AND PLASMA GENES IN *PARAMECIUM*

One of the most striking examples of cooperation between genes and particles in the cytoplasm (plasmagenes) occurs in *Paramecium*. The particle in the cytoplasm, composed largely of DNA, is known as *kappa*. (Since Arabic letters have been used to designate genes in the chromosomes, *a*, *b*, *c*, etc., the letters of the Greek alphabet are used to designate plasmagenes, *alpha*, *beta*, *gamma*, *delta*, etc.) The most thoroughly investigated of these is *kappa*. When present in sufficient amount in *Paramecium*, it produces a substance, *paramecin*, which is toxic to strains not possessing *kappa*.

The production and increase of *kappa* particles in the cytoplasm of *Paramecium* is dependent upon the presence of a dominant gene (*K*) in the nucleus. Animals must be *K/K* or *K/k* in constitution before *kappa* particles will increase. The *K* gene cannot initiate *kappa* production, but once a single *kappa* particle is introduced into a *K/—* genotype it will multiply and in sufficient numbers will produce the lethal substance, *paramecin*. Animals of this type are called killers. Recessive animals, *k/k*, are termed sensitive. The relationships are shown diagrammatically in Fig. 15-3. The different types of animals are shown in Table 15-2.

The *kappa* particle behaves very much like a virus that is capable of surviving only in animals that have at least one dominant gene *K*. Without *K*, the *kappa* particles disappear and the animal becomes a sensitive one.

Animals that have at least one *K* (*K/—*) are sensitive unless at least one *kappa* particle is introduced. If one *kappa* particle is introduced, and increases



After Sonneborn

Fig. 15-3 Cooperation between chromogenes and plasmagenes in *Paramecia*. Killer (left) has *kappa* particles in *K/—* genotype. Sensitive (right) lacks *kappa* particles. Unstable (center) lacks *K* gene and *kappa* disappears.

sufficiently, the *K/—* animal is transformed from a sensitive to a killer. It is possible for killer animals (*K/—*) to become sensitive if they reproduce very fast. The reproduction of the *kappa* particles may not keep pace with the reproduction of the nucleus and some animals may be produced with no *kappa* particles. They immediately become sensitive.

Table 15-2. Phenotypes Resulting from Different Combinations of Genotypes and "Plasmagenotypes"

Genotype	Plasmagenotype	Phenotype
<i>K/—</i>	<i>kappa</i>	killer
<i>K/—</i>	no <i>kappa</i>	sensitive
<i>k/k</i>	no <i>kappa</i>	sensitive
<i>k/k</i>	<i>kappa</i>	unstable (<i>kappa</i> disappears)

CYTOPLASMIC MALE STERILITY IN CORN

In Chapter 13 a male sterile condition in corn was discussed. Male sterile plants produce tassels, but with shriveled anthers and no pollen. This condition can be caused by any one of more than a score of different

genes. Possibly a great many more genes causing the male sterile condition could have been found in maize. However, investigators became engrossed with other interesting problems and almost completely gave up the practice of studying new mutants and locating them in linkage maps. During the first three decades of genetics (approximately 1901-1930), numerous investigators were discovering many genes in corn, studying their linkage relationships, and locating these genes in linkage maps. It was this pioneer work, led by R. A. Emerson at Cornell (and his students and associates), that made the maize plant the important genetic tool it is. In this period, the 10 chromosomes were mapped genetically. After 1930, some of this work was continued but the discovery and mapping of new genes proceeded more slowly.

So the number of 20 male sterile types conditioned by different genes is a conservative one. Possibly a great many more could be found if a diligent search were made.

In 1933 Rhoades, a student of Emerson and one of our eminent geneticists, discovered a new type of male sterility of corn. In this case the transmission was through the cytoplasm. Male sterile plants pollinated by normal gave male sterile plants entirely different from genic male steriles, which produce normal plants if pollinated by normal (since all of the genic male steriles are recessive).

The cytoplasmic male sterile of Rhoades, however, produced only male sterile plants when pollinated by normal plants. By contrast, all of the genic male sterile types gave results as shown in Table 15-3. It illustrates the performance of male sterile (ms_1) located in chromosome 6, close to the yellow-white locus governing the inheritance of yellow and white endosperm.

Table 15-3. Inheritance of Male Sterile, in Maize

$\begin{array}{c} \text{P} \\ \hline ms_1/ms_1 \times Ms_1/Ms_1 \\ \hline \text{F}_1 \\ \hline Ms_1/ms_1 \text{ fertile} \\ \hline \text{F}_2 \\ \hline 3 Ms_1/- : 1 ms_1/ms_1 \end{array}$			
<i>Testcross</i>			
Parental type	$\left\{ \begin{array}{l} y Ms_1/y ms_1 \\ Y ms_1/y ms_1 \end{array} \right.$	white seeds; fertile	725
		yellow seeds; sterile	657
Crossovers	$\left\{ \begin{array}{l} y ms_1/y ms_1 \\ Y Ms_1/y ms_1 \end{array} \right.$	white seeds; sterile	20
		yellow seeds; fertile	22
Total			1424

Percentage of recombinations 2.9

This male sterile gene shows about 3% crossing-over with the yellow-white locus (Singleton and Jones, 1930). Other genic male steriles have been reported in chromosomes 1, 3, 5, 8, and 9. It is possible that some may be in each of the ten chromosomes.

With the cytoplasmic male sterile, however, no linkage relations have been found with any of the ten pairs of chromosomes, since it is in the cytoplasm and not in the nucleus.

POLLEN RESTORERS OF FERTILITY IN MAIZE

The cytoplasmic male sterility in maize persists generation after generation in certain stocks. It is being used in the production of hybrid seed corn to eliminate detasseling. This work has been pioneered by Jones, inventor of the double cross described in Chapter 12. The method used is to cross a cytoplasmic male sterile by a desirable seed-parent type of inbred maize (for example Wf9), and then to backcross several generations to the inbred parent. This is illustrated in Fig. 15-4. Since the cross is made using pollen of Wf9 on the cytoplasmic male sterile (the only way it is possible to make the cross), the cytoplasm is male sterile and remains so during repeated backcrosses to the Wf9 inbred parent.

At the end of four or five generations of backcrossing, a new inbred is obtained, in which the nucleus is almost wholly from Wf9, and the cytoplasm is entirely from the cytoplasmic male sterile line. With Wf9 genes in male sterile cytoplasm, complete sterility is maintained. This makes Wf9 an ideal seed parent, because it produces no pollen and hence does not need to be detasseled. Even when crossed by another inbred (for example Indiana 38-11), the F_1 *ms* Wf9 \times 38-11 is a productive hybrid, also male sterile, and producing no viable pollen. It is an ideal seed parent and can be grown with no detasseling.

Students may ask why any commercial hybrid produced using this sterile hybrid as a seed parent will not be male sterile, too, since it will have sterile cytoplasm. When Jones first proposed this system of producing hybrid seed without detasseling, he suggested that such hybrid seed would have to be mixed with seed of the same hybrid produced by conventional methods, i.e., with normal cytoplasm capable of shedding pollen. If one fifth of the seed were of the conventional type, this should provide ample pollen source for the other four fifths, which would produce no pollen. Without the conventional type hybrid to shed pollen, the farmer growing the hybrid with male sterile cytoplasm would produce a bountiful supply of *cobs* with no kernels. He would not be happy about this.

In working with the male sterile cytoplasm and converting various inbreds to it, Jones made an interesting observation. When pollen of a few inbreds, for example Illinois Hy, was placed on silks of the cytoplasmic male sterile,

the F_1 hybrid was *fully fertile*. In some way the genes contributed by the inbred Hy had completely restored fertility to a plant whose cytoplasm would have produced a tassel with no pollen. Here, definitely, was a conflict of function. The cytoplasm called for a sterile tassel, whereas the genes of the Hy had a masking effect over the cytoplasmic male sterile.

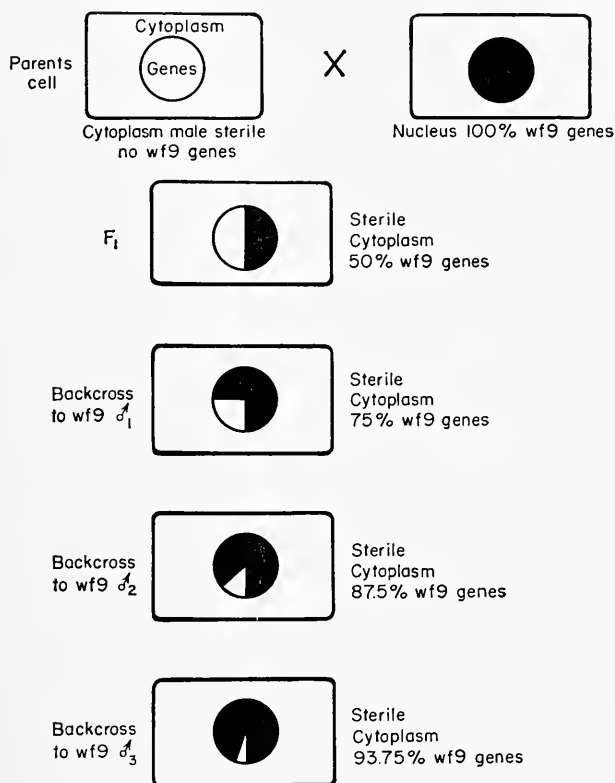


FIG. 15-4 Conversion of maize inbred (Wf9) with normal cytoplasm to one with sterile cytoplasm plus Wf9 genes. At the end of three backcrosses to Wf9, the resulting inbred has 90% Wf9 genes with sterile cytoplasm.

This is analogous to the gene-cytoplasm interaction in *Paramecium*, where the absence of gene *K*, i.e., in *k/k* animals, prevents the development and increase of the *kappa* particles.

Whether any particles similar to *kappa* are present in maize cytoplasm in sterile lines is not known, but the similarity between the two cases is striking. This is a good example of similarities in genetic behavior in widely different organisms, one a rather low form of animal life (a *Paramecium*) and the other one of the higher plants, a monocotyledon and a member of the grass family.

USE OF CYTOPLASMIC MALE STERILE AND POLLEN RESTORERS

The use of cytoplasmic male sterile plus the restorer genes of certain inbreds has made feasible the production of hybrid seed without detasseling. This not only saves thousands of dollars annually in the production of hybrid seed, but also assures seed of better quality. By this method it is possible to eliminate completely any outcrossing that occurs in a small percentage of cases in seed rows not properly detasseled. It is an excellent example of the contribution of the pure science of genetics to the practical problem of food production.

Scientifically, the elaboration of principles that can be used by the plant or animal breeder is no more important than an analysis in which practical application seems remote. The business of the researcher is to discover principles, whether or not they have immediate application. The research of Sonneborn and his colleagues in working out the cytoplasmic-genic relationships in *Paramecium* is fully as important as the work on the cytoplasmic genic relationships in *Zea mays*. In fact, the brilliant work in *Paramecium* enables us to understand more fully what is taking place in corn.

MATERNAL INFLUENCE

A number of cases have been studied of maternal influence, but not of cytoplasmic inheritance. Three of these will be cited: 1. Maternal influence in pigment in the European flour moth, *Ephestia*; 2. The "delayed" inheritance of coiling in snails; 3. The milk factor in cancer transmission in mice.

INHERITANCE OF KYNURENINELESSNESS IN EPHESTIA

A mutant was discovered in *Ephestia* that blocks the formation of kynurenine in the metabolism of tryptophane. The prevention of the formation of kynurenine in the flour moth has several phenotypic consequences. If the mutant is labeled k/k (for kynurenineless) and the normal K/K , the various phenotypic consequences of the mutant and the normal allele are shown in Table 15-4.

Since the mutant blocks the production of kynurenine that is essential to full pigment production, the result is a phenotypic expression in pigment formation in several parts of the body. This is truly a *pleiotropic* effect, where there are several phenotypic manifestations of a single gene difference. The gene k is in the nucleus, and the inheritance is not of a cytoplasmic nature as described for several examples earlier in this chapter. However, if a female is of the constitution K/K or K/k , a maximum amount of pigment develops in

Table 15-4. Genotypes and Phenotypes of Mutant k/k and Normals ($K/-$) in *Ephesia*

Character affected	Phenotype of K/K or K/k	Phenotype of mutant k/k
Adult eyes	black	red
Adult brain	dark brown	pale red
Adult testes	brown-violet	colorless
Larval skin	reddish	white
Larval eyes	much pigment	little pigment
Eggs produced	pigment	no pigment

the various areas of the body (Table 15-4). Some of this pigment is found in the eggs also, and the color persists for a time even in larvae hatched from k/k eggs. As the larvae grow, the color is used up. There is no more elaboration of kynurenine, so the larvae soon become white and all maternal effects are lost.

Since the color in the body of a female can be passed on to the eggs and consequently to the offspring, although in a transient phase, it is evident that there will be differences between reciprocal hybrids *in the larvae*, but not in adults. Any color passed on from a K/k mother to a k/k offspring has completely faded out by the time the larvae have metamorphosed into pupae and then adults. Hence adults with the *same genotype* all have the *same phenotype*. This is not true for larvae that show a strong maternal influence.

DELAYED SEGREGATION FOR COILING IN SNAILS

An interesting case of delayed segregation, although not one of cytoplasmic inheritance, is found in the fresh water snail, *Limnae*. There are two types of coiling of the shell, to the right (dextral) or to the left (sinistral). The determiner of this condition is apparently a gene, with right being dominant to the left. If the sinistral coiling is represented by s and dextral by S , the genotypes and phenotypes of reciprocal hybrids are as follows:

Parents	F_1 Hybrids
(1) $S/S \times s/s$	S/s dextral coiling
(2) $s/s \times S/S$	S/s sinistral coiling

Although the two reciprocal F_1 hybrids have the same genotype, they are different in their modes of coiling, one dextral and one sinistral. It seems that the coiling of the offspring is determined by the *genotype* of the mother. In the first cross the offspring are coiled dextrally because of the S/S condition of the mother. Conversely, in the second cross the coiling is all of the sinistral type because the mother was s/s .

What about the offspring of the two reciprocal hybrids? It is possible to

produce F_2 progeny by self-fertilization, since these snails are hermaphroditic. Since the genotype of both F_1 hybrids is S/s , and since S is dominant, we would expect that both reciprocal hybrids would show only dextral coiling in the F_2 (Table 15-5).

Table 15-5. Genotypes and Phenotypes for Coiling in Snails

P	
S/S (dextral)	\times s/s (sinistral)
F_1	
S/s (dextral)	
F_2	
Genotype	Phenotype
1 S/S	dextral
2 S/s	dextral
1 s/s	dextral (because mother was S/s)
F_3	
(Assuming each F_2 produced 4 offspring)	
1 S/S	4 S/S dextral
2 S/s	2 S/S dextral
	4 S/s dextral
	2 s/s dextral
1 s/s	4 s/s sinistral
Total F_3 progeny.....	16
$\frac{12}{16} =$ dextral; $\frac{4}{16} =$ sinistral	

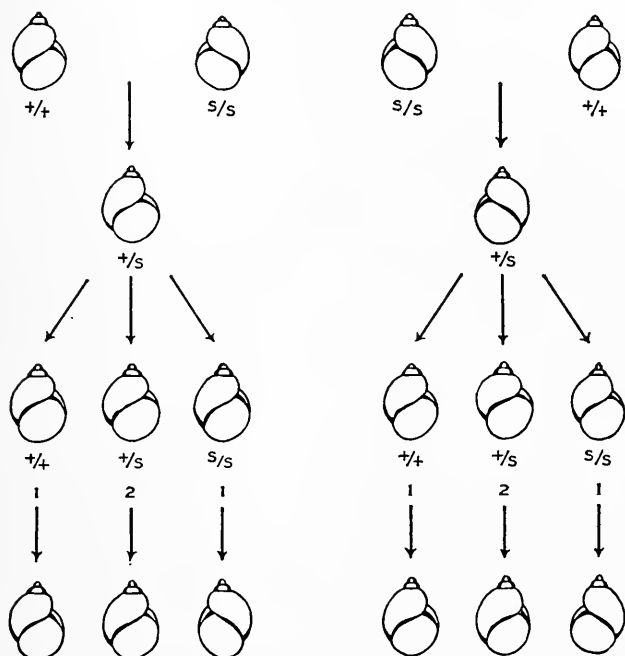
One fourth ($\frac{1}{4}$) of the F_3 progeny would be coiled to the left, showing a monogenic segregation which was delayed from F_2 to F_3 generation, since direction of coiling is determined by genotype of the mother. Thus we see that although the F_2 is all coiled dextrally (since the genotype of their mother was S/s , or dextral) the segregation occurs in the F_3 as a 3:1 ratio. This is illustrated in Fig. 15-5.

It should be emphasized that this is genic, not cytoplasmic, inheritance, but with the coiling of the progeny determined by the genotype of the mother. The example is an interesting case of delayed segregation.

MILK FACTOR IN CANCER TRANSMISSION IN MICE

J. J. Bittner (1940), in studying the transmission of susceptibility to breast cancer in mice, found that certain strains had a low susceptibility to

breast cancer, while others were prone to this disease. He also made an important discovery regarding the transmission of this disease. If mice from "resistant" strains were removed from their mothers at birth and placed with foster mothers of a susceptible strain, the resistant mice became susceptible and contracted mammary cancer about as frequently as the others.



Courtesy of Sturtevant and Beadle; and W. B. Saunders Co., Philadelphia

FIG. 15-5 Segregation of coiling in snails delayed from F₂ to F₃ generation.

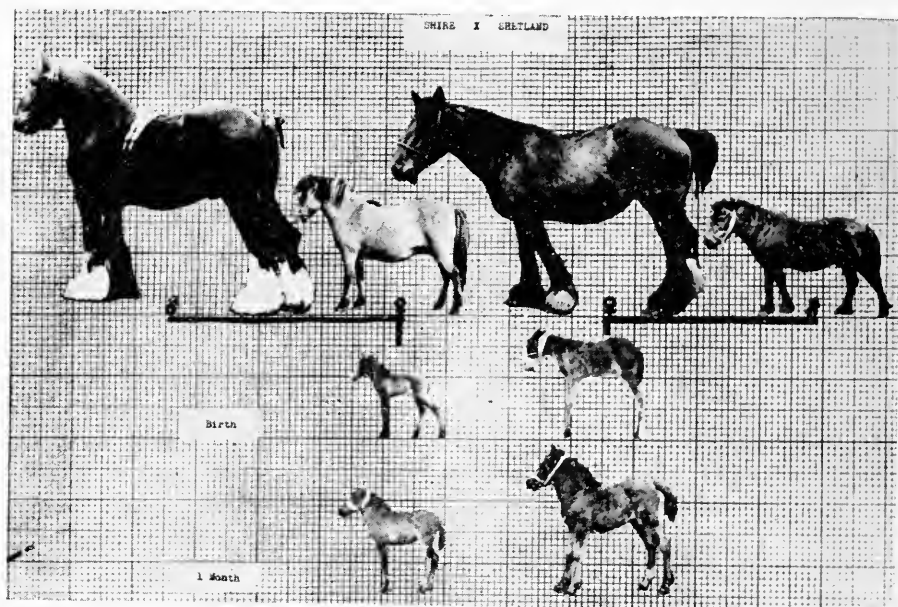
Conversely, if susceptible mice were removed from their mothers at birth and placed with foster mothers of a resistant strain, the susceptible ones became resistant and showed little incidence of the disease.

In this case the factor causing the disease seemed to be in the milk. Whether it is a virus is not known, but its behavior is similar to a virus, or another infective agent that is transmitted through the milk on which the young feed.

This is not a case of cytoplasmic inheritance. There are most likely genic differences between susceptible and resistant strains and they would be expected to follow the same laws as other genic differences. The study is an interesting one, well documented, of a maternal character that is transmitted through the mother's milk.

DIFFERENCES IN RECIPROCAL HYBRIDS IN HORSES AND IN CATTLE

A striking difference has been observed by Sir John Hammond in size differences in reciprocal hybrids in horses, also in cattle. He made a reciprocal cross between a Shire draft horse and a Shetland pony, by artificial insemination. The foal produced by the cross Shire ♀ × Shetland ♂ was much larger than the foal of the reciprocal hybrid (Fig. 15-6). This difference,



Courtesy of Sir John Hammond, Cambridge University, England

FIG. 15-6 Differences in reciprocal hybrids in horses. These probably are due to a combination of cytoplasmic inheritance, plus the cellular environment of the developing fetus. The foal from Shire ♀ × Shetland ♂ was much larger at birth than the reciprocal. Some difference in size persisted to maturity.

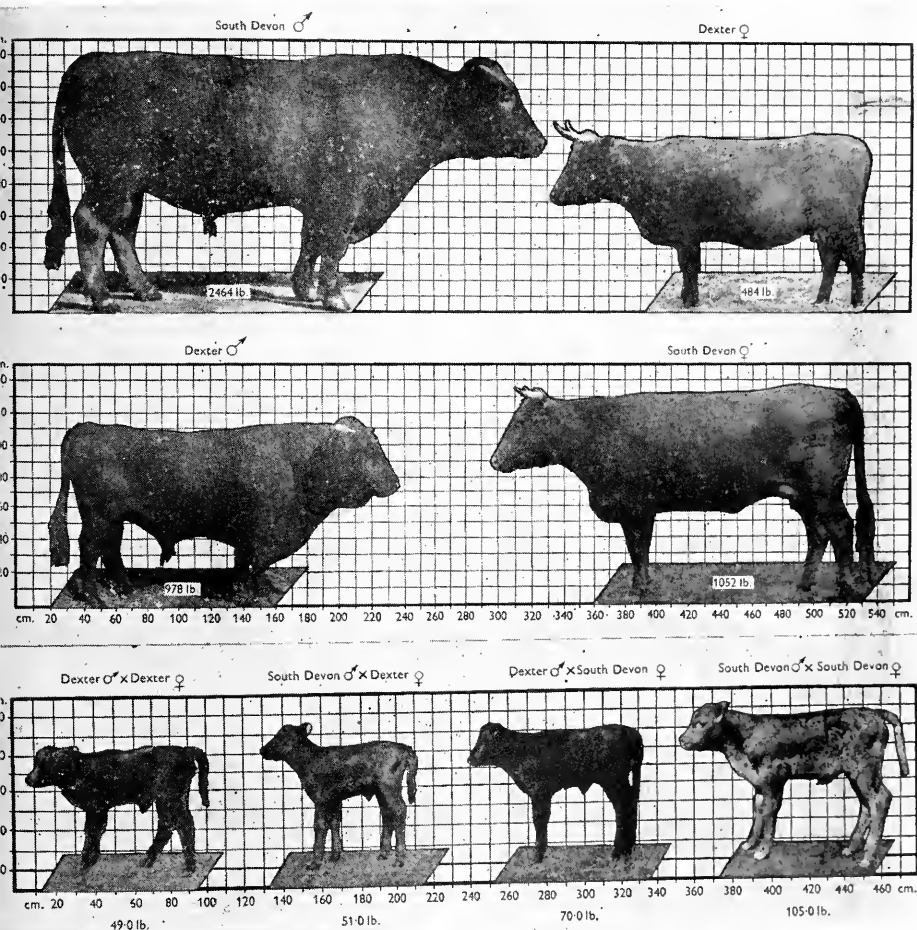
which persisted until maturity, could be attributed to cytoplasmic inheritance or to better nurture of the fetus in the larger mother. It is a striking example of a difference in reciprocal hybrids.

Another instance of this is the reciprocal hybrids in cattle between the Devon and Dexter breeds (Fig. 15-7).

CONCLUDING REMARKS

Although most observable phenotypic differences in plants and animals are determined by genes in the chromosomes, the cytoplasm, which is much more abundant, is essential to the well-being of the organism. The

cytoplasm is also used to transport material from which the DNA molecule of the nucleus is reduplicated. Also, the RNA in the cytoplasm is probably essential to the translation of the chromosome's genetic specification (contained in the DNA molecule) into action.



Courtesy of Sir John Hammond, Cambridge University, England

FIG. 15-7 Reciprocal crosses in cattle, Dexter ♀ produced smaller calves than Devon ♀. The largest calf was Devon ♀ x Devon ♂.

In addition, there are a few cases where the inheritance of characters is determined directly by the cytoplasm. This is true of some plastid inheritance and of the inheritance of cytoplasmic male sterility in certain plants.

In *Paramecium* the *kappa* particle is transmitted directly to stocks having the right genic constitution. In both *Paramecium* and maize there are interesting genic-cytoplasmic relationships.

In addition to characters inherited through the cytoplasm, three cases of maternal influence are cited: color in *Ephestia*, delayed segregation in snails, and the milk factor in the transmission of susceptibility or resistance to breast cancer in mice.

Striking differences in reciprocal hybrids in horses, and also in cattle, have been observed.

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PROBLEMS

- 15-1. Define or describe the following terms:

chromogene	iojap maize
cytoplasm	<i>kappa</i> (Paramecium)
cytoplasmic inheritance	kynurenineless (<i>Ephestia</i>)
cytoplasmic male sterility	male sterile (genic) maize
(maize)	maternal influence (<i>Ephestia</i>)
delayed segregation (snails)	milk factor in cancer transmission
deoxyribonucleic acid	plasmagene
DNA	plastid
fertility restorers (maize)	ribonucleic acid
gene reduplication	RNA
genic and plasmagenic	virus
interaction (Paramecium)	

- 15-2. Identify the following scientists, giving a major contribution, with approximate date:

Beadle, G. W.	Jones, D. F.
Bittner, J. J.	Rhoades, M. M.
Emerson, R. A.	Sonneborn, T. M.

- 15-3. The four o'clock has a variegated foliage. Some branches have all green leaves, some are pale, and some variegated with patches of green, pale,

and a mixture of green and pale. Fill in the spaces in the last column below, showing what types of plants would be grown from seed produced by different pollinations. The green color is in the plastids in the leaves, a part of the cytoplasm.

Branch supplying pollen	Branch with pollinated flowers	Colors of plants grown from seed
Green	Pale	
	-----	-----
	Variegated	
Pale	-----	-----
	Green	
	-----	-----
Variegated	Pale	
	-----	-----
	Variegated	
	-----	-----
	Green	
	-----	-----

- 15-4. A type of male sterility in corn is determined by the cytoplasm. This will be designated by the letter T (because of the fact that this particular strain came from Texas). Indicate whether the following cross will have fertile or sterile tassels:

$$T \times \text{inbred A} = ?$$

How would you produce inbred A with sterile tassels? If this inbred is then crossed to inbred B, the F_1 will produce tassels with no pollen. Tell how this sterile F_1 might be used in commercial seed corn production.

- 15-5. In maize there are several genes that produce male sterility. If you found a male sterile plant, how would you determine whether it was a genic or cytoplasmic male sterile?
- 15-6. In *Paramecium*, a cytoplasmic substance *kappa* produces a potent antibiotic that destroys any *Paramecium* not containing *kappa*. A gene in the nucleus *K* is necessary for the increase of *kappa* particles. What will be the phenotype of the following gene and cytoplasm combinations? Indicate by a check sign (✓) in the proper column.

Genotype	Cytoplasm	Killer	Sensitive	Unstable
$K/-$	<i>kappa</i>			
-----	-----	-----	-----	-----
$K/-$	no <i>kappa</i>			
-----	-----	-----	-----	-----
k/k	no <i>kappa</i>			
-----	-----	-----	-----	-----
k/k	<i>kappa</i>			
-----	-----	-----	-----	-----

- 15-7. Suppose you had a snail that showed dextral coiling. Upon self-fertilization this snail produced progeny that all showed coiling to the left (sinistral). Give a genetic explanation of this unusual behavior.
- 15-8. A heterozygous K/k female flour moth was mated to a male that was k/k . Two types of individuals would result from this mating. Give the genotypes of the two types with the phenotype of the eggs, larval eyes, larval skin, adult testes, adult brain.
- 15-9. Give the same information for the reciprocal mating.
- 15-10. The striping characters japonica (j) and iojap (ij) in maize are quite similar phenotypically. If you were given two seedlings, one japonica and the other iojap, how could you determine definitely which was japonica and which was iojap? What cross would you make? Give the two possible genotypes, also the genotypes and phenotypes of the F_1 plants and the expected genotypes and phenotypes of the F_2 .

Architectural Changes in the Chromosomes

MANY AND VARIED types of deviations from the normal chromosomal complement may affect the inheritance of one or more characters. Most higher plants and animals are diploid, having two sets of homologous chromosomes, one of which comes from the father and one from the mother. The gametes are haploid or monoploid, with half the number of chromosomes of the parent.

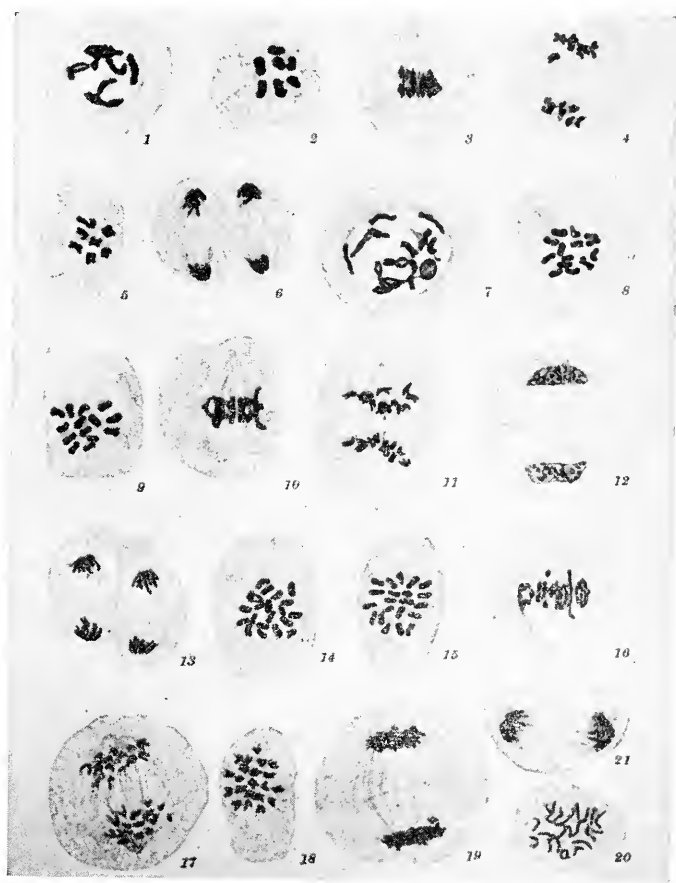
Changes in architecture of the chromosomes are of two types: variations in the total number of chromosomes, and alterations within the individual chromosome. Some changes involve not one, but two or more, chromosomes, in the way in which the material is arranged. This latter type is known as a translocation between two or more nonhomologous chromosomes and will be discussed later in the chapter.

Until the late 1920's or early 1930's, it was not possible to obtain full information about translocations and other chromosomal changes, because our precise knowledge about chromosome morphology was rather scanty. Two discoveries changed this materially—(a) McClintock found that all 10 maize chromosomes could be identified at mid-prophase, and (b) the researches of T. S. Painter and of Bridges showed that the bands in the salivary gland chromosomes of *Drosophila* could be identified with loci of known genes.

Let us first examine the gross architectural changes in chromosome number. Every plant and animal has its own characteristic number. For example, the pea plant studied by Mendel has seven pairs of chromosomes (or fourteen chromosomes in diploid tissue), the corn plant has ten pairs, and man twenty-three.

POLYPLOIDY IN PLANTS

Many plant species within a given genus have chromosome numbers that are multiples of a basic number. A good example is the genus *Triticum* (wheat). The most primitive species has seven pairs, while other species have fourteen and twenty-one chromosome pairs, respectively (Fig. 16-1).



Courtesy of Karl Sax; and Genetics

FIG. 16-1 Chromosome numbers of three wheat species, *T. monococcum* $n = 7$ No. 1-5, *T. durum* $n = 14$ No. 8-9, and *T. vulgare* $n = 21$ No. 14-20.

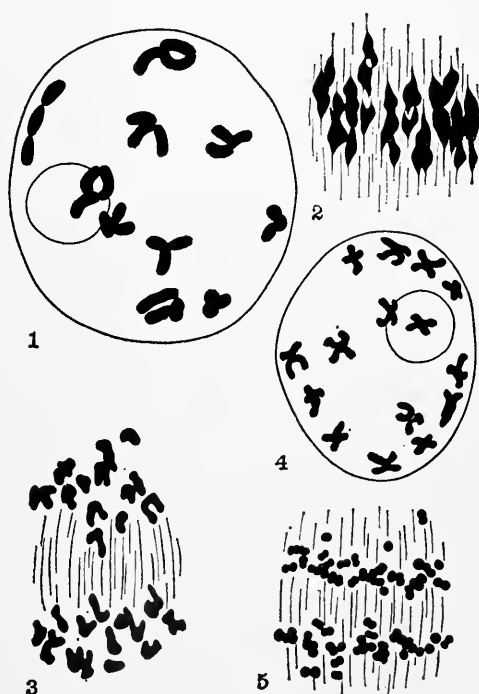
As a general rule the species with the higher chromosome numbers are regarded as more advanced from an evolutionary standpoint than those with the lower numbers. It is thought that the plants with the higher numbers have evolved from those with the lower, either by a direct increase of the lower numbers, or by an increase after crossing with other species. The first type of increase is known as *autopolyploidy*, while the second is known as *amphidiploidy*.

The chromosome numbers of the wheat species were worked out by Karl Sax, a student of E. F. Gaines, geneticist and wheat breeder, at Washington State College, Pullman. Later he was a graduate student of East at the Bussey Institution of Harvard University. The chromosome numbers of the various wheat species as found by Sax (1922) are given in Table 16-1.

Table 16-1. Chromosome Number of the Wheat Species

Species	Chromosome Number	
	n (gametes)	2n (somatic tissue)
<i>Triticum monococcum</i>	7	14
<i>Triticum durum</i>	14	28
<i>Triticum vulgare</i> (aestivum)	21	42

T. monococcum is often called a diploid, *T. durum* a tetraploid, and *T. vulgare* a hexaploid. These names are commonly used, but they do not describe exactly the cytology of the different species. With such a naming system, one might conclude that a species with 28 chromosomes has four sets of seven



Courtesy of L. F. Randolph and Barbara McClintock;
and American Naturalist

FIG. 16-2 Chromosomes of triploid maize plant with ten sets of three chromosomes.

chromosomes each because it was produced by doubling the number of a 14 chromosome species. This might be true. Many polyploidizing agents are now available. If it is true, the 28 chromosome species is an autotetraploid. To be sure of it, a morphological study of the chromosomes must be made. But it may *not* be true. There are other ways of increasing chromosome numbers, as will appear presently. The student should be aware, however, that the name tetraploid or hexaploid does not necessarily mean four or six sets of homologous chromosomes.

On occasion a triploid plant arises which has, instead of a certain number of pairs, the basic species number of sets of three each. Such a triploid maize plant with ten sets of three chromosomes each at meiosis was reported by Randolph and McClintock (1926); see Fig. 16-2. This proved to be a fortunate occurrence, in that the progeny of this triploid plant enabled McClintock and Hill (1931) to associate different linkage groups with specific chromosomes, identified by their morphology.

The cultivated bread wheat, *T. vulgare*, with 21 pairs of chromosomes, did not arise from a doubling of the chromosomes of *T. monococcum* (autopolyploidy). Rather it was evolved from crosses of *T. monococcum* with sets of chromosomes from wild grasses, *Aegilops* sp., relatives of wheat. Possibly whole sets of seven chromosomes from *Aegilops* were combined with the set of seven chromosomes from *T. monococcum*. Each set of such chromosomes is known as a *genome*. Geneticists and cytologists in recent years have done extensive research in which a study of genomes has contributed to an understanding of the evolution of many plant species.

INDUCED POLYPLOIDY IN PLANTS

Early in 1937, O. J. Eigsti observed some polyploid cells in onion root tips he had treated with colchicine (Fig. 16-3). The drug is the same one used since the time of the Pharaohs in the treatment of gout, a metabolic disturbance of man. It is still employed medicinally to alleviate acute symptoms, and small daily doses are given to prevent acute attacks. The dose for medicinal use is small—approximately .0005 g. per day, or about one part in 15 million per body weight of an average man. How such a small amount can account for such a large effect is not known. This will be discussed further in Chapter 19 on biochemical genetics in man. The concentrations to induce polyploidy are much higher, at least .05%, or five parts in 10,000 (500 p.p.m.).

The introduction of colchicine to induce chromosome doubling initiated research by many plant investigators. In fact, it started what was known as the "colchicine fad." Later researches have shown that the chemical is not a cure-all for the problems of the plant breeder, but a useful tool for the production of polyploidy, with concomitant increase in vegetative and floral parts.

Where an increased floral size has commercial possibilities, the use of



*Courtesy of O. J. Eigsti and P. Dutton;
and Iowa State College Press, Ames*

FIG. 16-3 Polyploid cells induced by colchicine in onion root tips, the first polyploid cells reported induced by colchicine. (A) untreated; (B) treated; and (C) photomicrograph of section from treated root. (A) Roots grown in tap water do not show enlargement. (B) Colchicine solution of 0.01% causes spears, or colchicine-tumors.

colchicine has proved a boon to the plant breeders. An example of an Easter lily with larger flowers, produced by Samuel L. Emsweller of the United States Department of Agriculture, is shown in Fig. 16-4.

MONOPOID OR HAPLOID PLANTS

The basic chromosomal number of any series of polyploids is the monoploid (or haploid) number of the most primitive species in the group, i.e., the one with the lowest chromosome number. In rare instances, haploid or monoploid plants occur with the gametic instead of the usual somatic chromosome number. For example, in corn plants with ten pairs (at meiosis) of chromosomes (twenty in somatic tissue), small plants are occasionally found with only ten chromosomes in somatic tissue. The root tip, with many mitotic

divisions, is the conventional material for the determination of somatic chromosome numbers.

Monoploids will grow to maturity even though they possess only half the normal number of chromosomes. They probably survive because of *chromo-*



Courtesy of S. L. Emsweller; and the
U. S. Department of Agriculture

FIG. 16-4 Diploid and tetraploid Easter lilies.

somal balance. It is known that genetic types lacking a single chromosome may be rather low in viability, with many being nonviable.

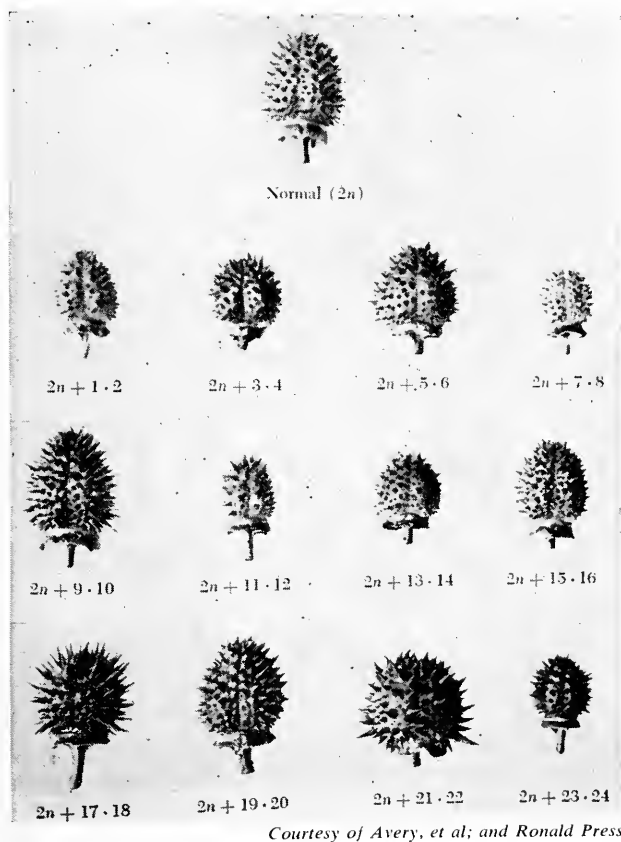
POLYSOMIC CHROMOSOME NUMBERS

In addition to showing variation of complete sets of chromosomes (genomes), individuals may have only one chromosome extra, or be deficient for a single chromosome. In corn, for example, a normal plant has twenty chromosomes (ten pairs at meiosis). A *trisomic* plant has the normal complement of twenty chromosomes, plus one extra. A *monosomic* has one chromosome missing. These plants with less than a complete set of chromosomes extra or deficient are known as *aneuploids*, in contrast to the normal condition, where each chromosome present has a homologous partner (*euploids*). At meiosis of trisomics, some cells have the extra chromosome loosely associated with a particular pair. Other cells may show the extra one free in the nucleus. In corn with ten pairs of chromosomes, it is evident there can be ten different trisomics, each having a different chromosome as the extra one, all of which have actually arisen from the progeny of a triploid plant.

TRISOMICS IN DATURA

The work of Blakeslee, Belling, and their associates in establishing 12 different trisomics is one of the classic experiments of genetics. This is summarized by Blakeslee (1934). *Datura stramonium* (Jimson weed) has

twelve pairs of chromosomes at meiosis, 24 in somatic tissue. Blakeslee, Belling, and associates were able to isolate 12 different trisomies in the study of a triploid plant ($3n$). These 12 different trisomies were distinguishable morphologically by the size, shape, and marking of the fruiting bodies, the seed pods



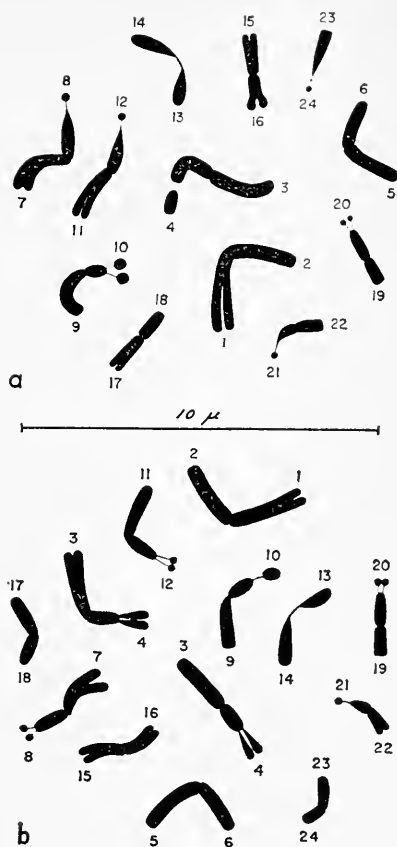
Courtesy of Avery, et al; and Ronald Press

FIG. 16-5 Phenotypic expression in seed pods of twelve different trisomies in *Datura*, in comparison with the normal.

(Fig. 16-5). The chromosomes in the pollen of one of these trisomies, as well as those of a monoplod plant, are shown in Fig. 16-6.

TRISOMICS IN MAN

In man it has recently been demonstrated cytologically that the severely retarded condition known as Mongolism is determined by the addition of an extra autosome to the normal complement of 46 chromosomes. One of the smaller autosomes is represented in triplicate rather than in duplicate, as normally found. Mongolism is much more frequent among the chil-



Courtesy of Avery, et al.; and Ronald Press

FIG. 16-6 (a) Chromosomes of a monoploid (haploid) in *Datura*. Each chromosome morphologically distinct from the others. (b) Chromosomes in pollen of a trisomic *Datura* plant with extra 3-4 chromosome $2n + 1$.

dren of older women. Possibly nondisjunction of the chromosomes happens more often as women grow older.

NULLISOMICS IN WHEAT

A *nullisomic* is an individual with *both* of the chromosomes of any given pair missing. Sears (1944), working with *Triticum vulgare*, a bread wheat, has made a rather extensive study of nullisomics. He isolated and identified 17 of the possible 21 nullisomics. In most organisms nullisomics are nonviable.

Trisomics are found in wheat also, but are hard to identify, because phenotypically they are so similar to normal wheat plants with the diploid chromosome number.

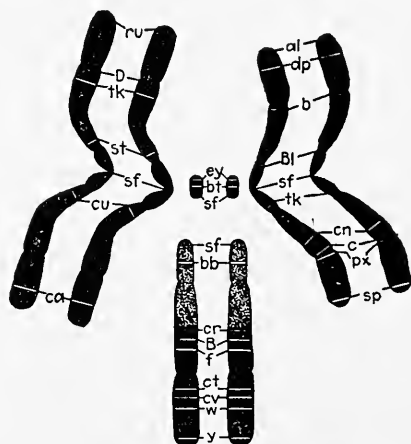
Two other types of "heterosomics" should be mentioned. One is the *tetrasomic*, which results when a chromosome is represented by four, rather than two as in disomics or diploids, or by three in the case of trisomics. The other is the double trisomic in which two *different* chromosomes each has one extra. Both of these types have been found in *Datura*. Also types are found in *Datura* with an extra "synthetic" chromosome made up of the two short or long arms of a single chromosome. These are known as secondary trisomics.

CYTOLOGICAL IDENTIFICATION OF CHROMOSOMES

This heading is not meant to apply to the observation of the chromosomes in order to identify them as chromosomes, but rather to the morphological distinction between the different chromosomes, so that each could be positively identified. Two big advances in this field, mentioned earlier, came in the late 1920's and early 1930's. The first was the brilliant research of McClintock, who for the first time was able to identify each of the ten pairs of chromosomes at mid-prophase of the first meiotic division (pachytene). Once the identity of the ten pairs of chromosomes was known, the next step was to associate each of the ten visible chromosomes with a known linkage group. McClintock naturally became the leader in this field and the first cytogeneticist of maize.

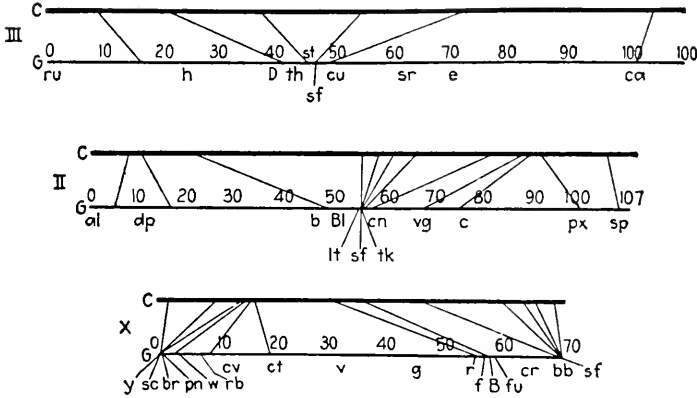
The second big advance was the research of Painter (1934) that established the extremely large salivary gland chromosomes in *Drosophila* as an important cytogenetic tool. (It had been known for many years that, in some of the Diptera, the salivary gland chromosomes were unusually large.) Painter did not discover the *Drosophila* salivary gland chromosomes, but with excellent technique he was able to see many bands across them. These he assumed to be the loci of different genes.

The researches of many investigators since then have confirmed the supposition. *Drosophila* was then changed immediately from a cytologically mediocre organism to one of the best.



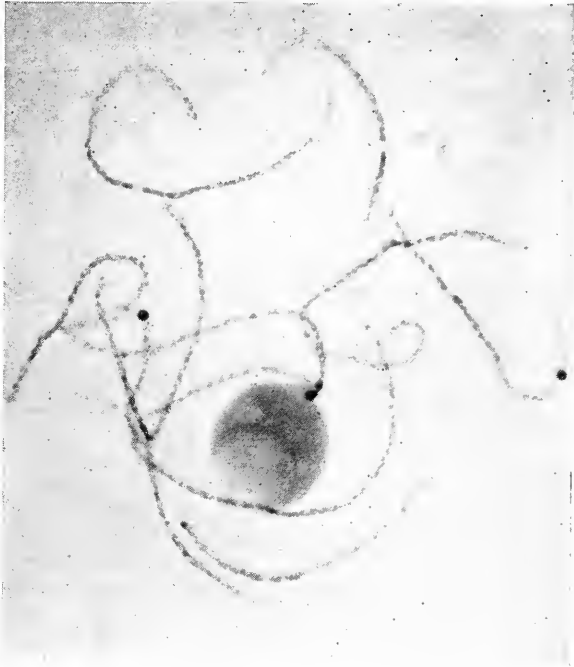
Courtesy of Th. Dobzhansky; and McGraw-Hill Book Company

FIG. 16-7 Dobzhansky's cytological map of somatic chromosomes of *Drosophila*, showing location of several genes in each chromosome and of the spindle fibers (*sf*). The inert region of the X chromosome is represented by the stippled portion of the rod-shaped chromosomes. The longer V-shaped chromosomes (left) are the third chromosomes; the shorter V-shaped chromosomes (right) are the second chromosomes; the smallest pair, the fourth chromosomes.



Courtesy of Th. Dobzhansky; and the McGraw-Hill Book Company

FIG. 16-8 Comparison of cytological maps (C) and genetic maps (G) for X chromosomes and autosomes 2 and 3 in *Drosophila*.



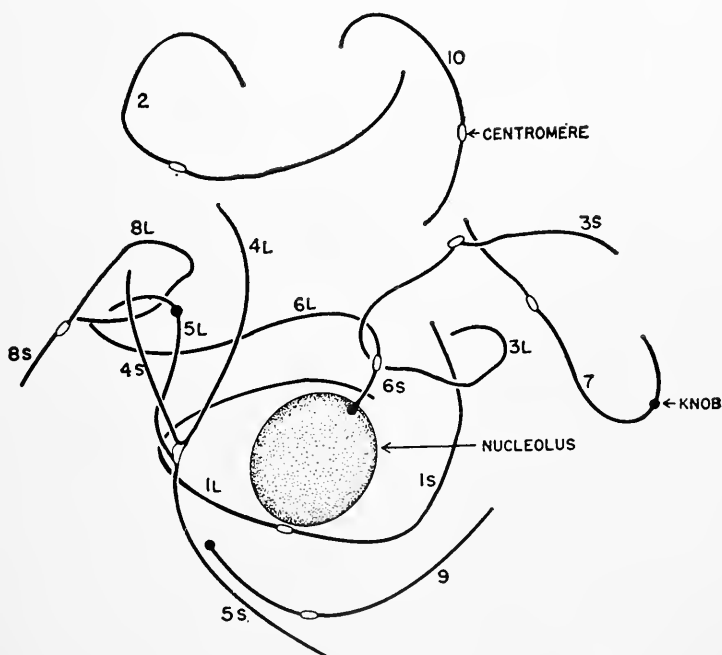
Courtesy of D. T. Morgan, Jr., M. M. Rhoades; and *Journal of Heredity*

FIG. 16-9 Ten chromosomes of maize, in pachytene.

Before the investigation of salivary gland chromosomes in *Drosophila*, considerable was known about the cytology of the fly. It had been established that the sex-linked genes were associated with the heteromorphic XY pair, and it was also known that the fourth chromosome was associated with the smallest linkage group.

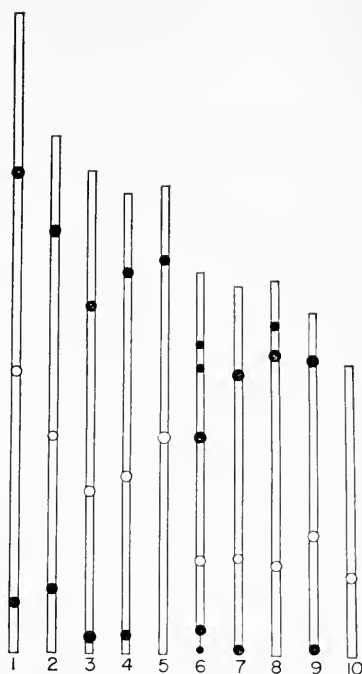
Dobzhansky had constructed cytological maps of the metaphase chromosomes, showing the approximate location cytologically of a number of genes in all of the chromosomes. Many of these genes were located by their associations with places where the chromosomes had been broken in reciprocal translocations—to be discussed shortly. Although the metaphase chromosomes are small, Dobzhansky succeeded, by dint of much labor, in the task of mapping them.

Dobzhansky's cytological map of *Drosophila*, published in 1936, is shown in Fig. 16-7, and a comparison of his genetic and cytological maps in Fig. 16-8. The genes in both show the same order, but the relative distances on the two maps do not always agree, indicating a greater concentration of genes in some portions. This was confirmed later in salivary chromosome maps. Some regions of the chromosomes have very few genes. For example, the Y chromosome is essentially devoid of hereditary markers and is composed mostly of *heterochromatin*, in contrast to other chromosomes with abundant genes.



Courtesy of M. M. Rhoades; and *Journal of Heredity*

FIG. 16-10 Ten chromosomes of maize, showing centromeres and most common knobs.



Courtesy of M. M. Rhoades;
and *Journal of Heredity*

FIG. 16-11. Description of distinguishing morphological characteristics of ten maize chromosomes (after Longley). The knobs (solid circles) are shown in their characteristic locations. Some strains of maize have chromosomes lacking in knobs while other strains have many knobs. The centromeres are represented by clear circles. To aid in the identification of the different chromosomes, the following comments call attention to important landmarks.

CHROMOSOME 1: Longest of complement. Knob or enlarged chromomere near end of short arm, and an enlarged chromomere at end of short arm. Average length at mid-pachytene is 82.40 micra. Ratio of long arm to short arm is 1.3:1.0.

CHROMOSOME 2: Sometimes difficult to distinguish from No. 5 but differs from the latter in ratio of arm lengths. The knob in long arm of No. 2 is farther from the end than is the knob in long arm of No. 5. The regions adjacent to the centromere of No. 2 are more pycnotic than is the case in No. 5. Length is 66.50 micra at pachytene. Ratio of long:short arm is 1.25:1.0.

CHROMOSOME 3: Ratio of arms 2:1. Knob in long arm lies approximately in middle of arm. Some strains have a knob near the end of short arm. Length at pachytene is 62.00 micra. Ratio of long:short arm is 2.0:1.0.

CHROMOSOME 4: Knob or enlarged chromomere near end of short arm. Differentiated from No. 3 by ratio of arm lengths. Pachytene length is 58.78 micra. Arm ratio is 1.6:1.0.

CHROMOSOME 5: Arms of almost equal length. Knob in longer arm. Pachytene length is 59.82 micra. Arm ratio is 1.1:1.0.

CHROMOSOME 6: Attached to nucleolus by deep-staining nucleolar organizing region. Length at pachytene 48.73 micra. Arm ratio is 7.1:1.0.

These areas in which many genes reside are known as *euchromatin*. The X chromosome has a rather long inert region near the centromere. This is also heterochromatin.

McCLINTOCK'S MORPHOLOGY OF MAIZE CHROMOSOMES

Let us return briefly to the classic research that was the beginning of the cytogenetics of maize.

McClintock's finding that the ten pairs of chromosomes in maize were morphologically distinguishable was the discovery necessary for the cytological and genetical rapprochement in maize (Figs. 16-9 and 16-10). It was then possible to determine which linkage group was located in which chromosome. She learned that the first linkage group established in maize, the *c sh wx* group, was located in the short arm of chromosome 9, the next to the shortest.

The ten pairs of chromosomes in maize were distinguished by three different criteria:

1. The length, with chromosome 1 more than twice the length of chromosome 10.
2. Ratio of the short arm of the chromosome to the long arm, in relation to the centromere. In chromosome 5 the length of the short arm is approximately equal to that of the long arm, with a ratio of 1.1:1. In chromosome 6, one attached to the nucleus, the ratio is 7.1:1.
3. Morphological features, such as knobs and deeply staining regions, especially around the centromere, that give different chromosomes a characteristic look. For example, chromosome 10 has a deeply staining region near the centromere, with a thin tapering region near the end of the long arm.

The best concise description of the 10 pairs of maize chromosomes appears in a publication by Rhoades (1950), who states he has "drawn rather heavily upon certain publications of my colleagues Longley, Burnham, Randolph, Beadle, Anderson, and others, but above all, on those of McClintock." With Rhoades' permission, we are reprinting a diagram (Fig. 16-11) showing the

CHROMOSOME 7: Region of long arm adjacent to centromere is very deep staining. Occasionally a strain is found with a knob at end of short arm. Length is 46.78 micra. Arm ratio is 2.8:1.0.

CHROMOSOME 8: Marked disparity in arm lengths. Regions on both sides of centromere are pycnotic. Length is 47.48 micra. Arm ratio is 3.2:1.0.

CHROMOSOME 9: Terminal knob at end of short arm. Pycnotic region, occupying proximal third of short arm. Length is 43.24 micra. Arm ratio is 1.8:1.0.

CHROMOSOME 10: Shortest of complement. Short arm has distinctive chromomere pattern with deep-staining chromomeres next to centromere and small tapering chromomeres at end. Length is 36.93 micra. Arm ratio is 2.8:1.0.

length, centromere location, and the knobs, or deeply staining bodies, of the 10 chromosomes found in certain genetic stocks.

ASSOCIATION OF LINKAGE GROUPS WITH SPECIFIC CHROMOSOMES

Various methods may be employed in identifying certain chromosomes with known linkage groups. One involves the use of trisomics. Since the extra chromosome can be any one of the chromosomes from one to ten, it is apparent that there can be ten different trisomics in maize. Fortunately, Randolph and McClintock (1926) found a plant that was triploid, having three sets instead of two, or a total complement of 30 chromosomes. Among the descendants of the original triploid plant, it was possible to isolate 10 different trisomic plants, each with a different extra chromosome in addition to the normal diploid complement of twenty. The first of the trisomics to be studied intensively, both cytologically and genetically, was the trisomic for chromosome 10. (McClintock and Hill, 1931.)

This study concerned the shortest chromosome (10). However, an appendix was added, stating that, while the report was in press, three other linkage groups had been positively identified with different chromosomes, and that the independence of six of the ten linkage groups had been definitely established.

TRISOMIC INHERITANCE OF R/r ALLELES

The R/r gene for anthocyanin production (in the presence of A and C) was the one used by McClintock and Hill in studying trisomic inheritance. With only two alleles at this locus, an R/r plant upon selfing produces a ratio of three colored kernels ($R/\text{—}$) to one colorless (r/r), or 1 R/r : 1 r/r , when testcrossed. If, however, there are three chromosomes instead of two, a heterozygous individual could be either $R/R/r$ or $R/r/r$. Let us see the consequences of segregation of these two genetically different trisomics. It must be remembered that, although there are three chromosomes, there are only two poles of the cell to which these chromosomes can go. Consequently one nucleus will get one No. 10 chromosome at meiosis, the other nucleus two (Table 16-2). One of the R genes is labeled R' (R prime).

Table 16-2. Distribution of R , R' , and r Genes in Chromosome 10, in Corn Plant Trisomic for Chromosome 10

CHROMOSOME (AND GENE) DISTRIBUTION

One Nucleus	Other Nucleus
R	$R'r$
R'	Rr
r	RR'

Assuming equal viability of all of the gametes, a testcross of such a plant would give a phenotypic distribution of 5 R : 1 r .

The genotype $R/r'/r$ would give a similar distribution (Table 16-3).

Table 16-3. Distribution of R , r' , and r Genes in Chromosome 10, in Corn Plant Trisomic for Chromosome 10

CHROMOSOME (AND GENE) DISTRIBUTION

One Nucleus	Other Nucleus
R	$r'r$
r	Rr'
r'	Rr

LOW VIABILITY OF POLLEN WITH EXTRA CHROMOSOME

In the distributions given in Tables 16-2 and 16-3, half of the potential gametes have an extra chromosome. Are these fully viable? It has been found that very few of the pollen grains (1—2%) carrying the extra chromosome function in competition with normal pollen grains. Since the percentage is so low, the $n + 1$ pollen grains can be virtually ignored in interpreting ratios.

On the pistillate side, the selection against eggs carrying 11 chromosomes is not as rigid as in the case of pollen grains. With no selection, one half of the eggs would be expected to have 10 chromosomes, the other half, 11. Actually, instead of a 50:50 distribution it has been found that two thirds of the eggs carry 10 chromosomes, to one third with 11. In a total of 124 plants examined cytologically by McClintock and Hill from a cross of $2n + 1 \text{ } \varphi \times 2n \text{ } \delta$, there were 83 plants with $2n$, and 41 with $2n + 1$ (33%). When an $r/r \text{ } \varphi$ was crossed by a $R/R/r \text{ } \delta$, they found a total of 646 R : 355 r , a good fit to a 2:1

Table 16-4. Ratio of R and r Kernels Resulting from Pollination of $R/r \times R/R'/r$ ($n + 1$ pollen grains rarely function)

φ gametes \searrow σ^7 gametes	R	R'	r
R	$R \ R$	$R \ R'$	$R \ r$
r	$R \ r$	$R' \ r$	$r \ r$

Phenotypes produced
 5 R — (colored)
 1 r/r (colorless)

ratio, which is expected if the $n + 1$ pollen grains rarely function (see Table 16-2 for gametes formed by $R/R/r$ plant).

When an R/r plant was pollinated by one that was $R/R/r$, a ratio of approximately 5 R : 1 r was obtained—2102:435. This is diagramed as in Table 16-4, which shows why a 5:1 ratio is expected when an R/r plant is pollinated by $R/R/r$.

NO TRISOMIC INHERITANCE IN OTHER LINKAGE GROUPS

McClintock and Hill planned experiments to demonstrate that the abnormal inheritance of the anthocyanin color in the kernel (R) was conditioned by the extra No. 10 chromosome, which they observed cytologically. It was necessary to show that this extra chromosome had *no effect* on the inheritance of characters located in the other nine chromosomes.

To test this hypothesis, McClintock and Hill studied the inheritance of characters located in eight of the other nine chromosomes to see whether they all showed disomic inheritance, or whether there might be trisomic inheritance, as found for the R gene. It was expected that disomic inheritance would be found for all characters not located in chromosome 10. This turned out to be true for eight of the other nine chromosome pairs. A test was not made for chromosome 8, which has few genetic characters. The characters studied are listed in Table 16-5.

Table 16-5. Characters Showing Disomic Inheritance in Trisomic ($2n + 1$) Plants, with Chromosome 10 Present in Triplicate

Chromosome	Character	Gene Symbol
1	Pericarp color, brachytic plant	P, br
2	Nonintensified plant color	b
3	Dwarf, anthocyanineless	d, a
4	Sugary (sweet)	su
5	Red versus purple aleurone	pr
6	White seed	y
7	Glossy seedling	gl
8	—	—
9	Aleurone color, waxy starch	C, wx

It is not necessary to give in detail the experimental results of all of these studies. One example, the inheritance of Su/su , will be presented. A homozygous sugary su/su plant, which was trisomic for chromosome 10, was crossed by a homozygous $2n$ starchy Su/Su . The F_1 plants, $2n$ and $2n + 1$, were self-pollinated and testcrossed for trisomic or disomic inheritance (Table 16-6). Both of these clearly indicate a disomic ratio. Had the first plant been

Table 16-6. Ratio of *Su* and *su* Kernels in 2n Plants and in Plant Trisomic for Chromosome 10

Plant Selfed	Genotypes	% <i>su</i>
2n + 1 <i>Su/su</i>	1758 <i>Su</i> /— : 586 <i>su/su</i>	25.0%
2n	1934 <i>Su</i> /— : 633 <i>su/su</i>	24.7%

trisomic for chromosome 4 (*Su/su/su*), an approach to a 2:1 ratio instead of a 3:1 would have been expected, as explained previously. Also a 2:1 ratio might have resulted from a cross of *Su/su* by *Su/su/su*. Actually the ratio from such a pollination was 1495 *Su* : 548 *su*, a close approximation of a 3:1 ratio (26.8%).

The same disomic type of inheritance was observed for characters located in eight of the nine linkage groups, other than linkage group 10. Consequently trisomic inheritance was well established for a character (*R*) located in chromosome 10 which had three, instead of two, No. 10 chromosomes. The extra No. 10 chromosome had no effect on the inheritance of characters in linkage groups other than 10.

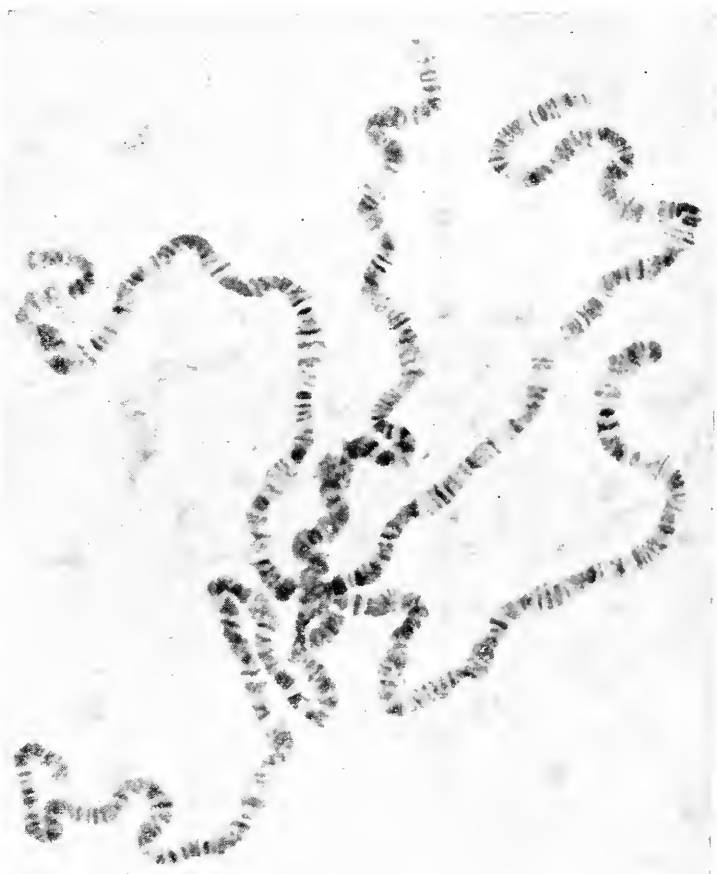
This experiment has been cited in some detail because it established with such precision the cytological basis for the inheritance of a particular character.

SALIVARY GLAND CHROMOSOMES IN DROSOPHILA

The giant salivary gland chromosomes of *Drosophila* are now familiar to all beginning students in biology. We have grown so accustomed to their use that we have forgotten the excitement caused when Painter's research in the early 1930's first called attention to them and their usefulness in the cytogenetics of *Drosophila*. The tremendous advance in analysis of complex chromosomal rearrangements would have been virtually impossible otherwise. Fig. 16-12 shows the salivary gland chromosomes of *Drosophila*, with deeply staining bands that correspond to the loci of known genes. This is from a slide of Berwind P. Kaufmann, one of the leading cytologists of *Drosophila*, and is published with his permission.

The chromosomes in the salivary glands are much enlarged. It seems that the chromosomes have divided many times, without separating, so that they consist of many strands, instead of the two- and four-strand stages at meiosis. Since there are many strands, the deep staining chromomeres take on the appearance of a band extending across the whole chromosome.

How does the heterochromatin appear in the salivary gland chromosomes? It is known that the Y chromosome in male *Drosophila* is largely heterochromatin, and so are a sizable portion of the X chromosome near the centromere, and also much of the small fourth chromosome. It has been observed



Courtesy of B. P. Kaufmann; and *Journal of Heredity*

FIG. 16-12 Salivary gland chromosomes of female larva of *Drosophila melanogaster*.

that in the salivary gland chromosome *all* of the heterochromatin forms a "chromocenter" from which the euchromatic arms of the chromosomes radiate. This can be seen clearly in Fig. 16-12.

Our precise knowledge regarding various chromosomal anomalies, such as deficiencies (or deletions), duplications, and reciprocal translocations was made possible by the study of the salivary gland chromosomes in *Drosophila*, as well as pachytene chromosomes in maize. The anomalies will now be discussed.

DEFICIENCIES (DELETIONS)

Perhaps the simplest type of anomaly within a chromosome is a deficiency, where the chromosome has a short segment missing. The most

common type is an internal one. Here, the missing part does not involve the end of the chromosome.

A deficiency for a considerable portion of a chromosome is shown in the heterozygous condition in Fig. 16-13. It can be observed that two homologous parts of the chromosomes pair, leaving the nondeficient portion of the chromosome unpaired, and forming a loop or a bulge. If any genes were in the missing segment (c and d in Fig. 16-13), the alleles of these genes will be expressed in a "hemizygous" condition.

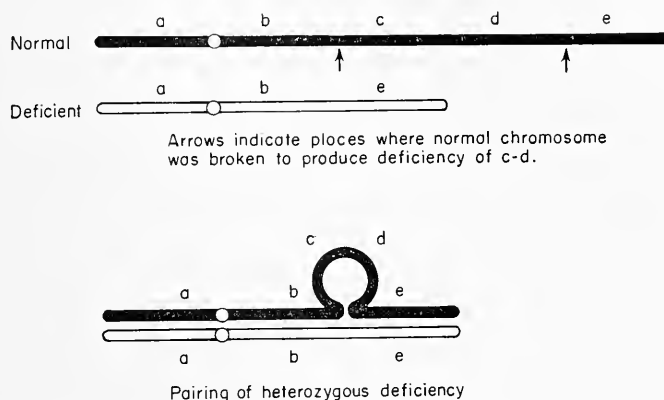
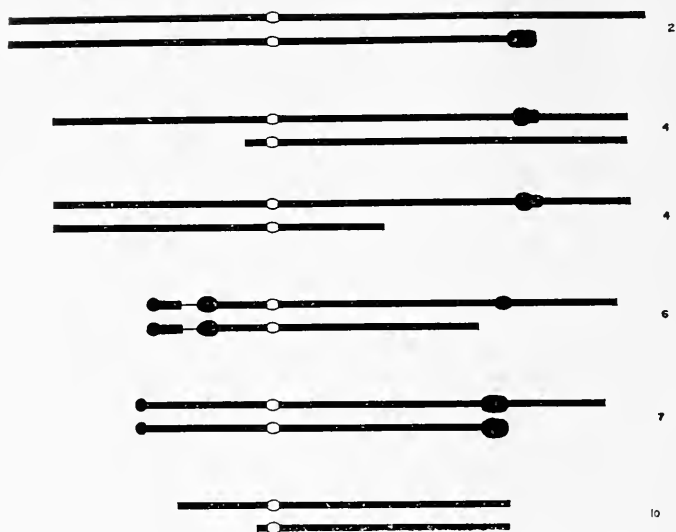


FIG. 16-13 Heterozygous deficiency for a portion of chromosome. Note the loop formed because of the portion of chromosome with no homologous portion in the homologue.

In fact, this is one way of positively identifying a linkage group with a known chromosome. In corn, if the pollen is placed on the silks of a recessive stock, most of the F_1 plants produced have the phenotype of the dominant allele. The rare exception showing the recessive phenotype may be suspected of being deficient for a portion of chromosome carrying the dominant allele. Examination of the recessive type may reveal which of the ten chromosomes has a portion deficient.

This technique was utilized by the author in a study of ultraviolet-induced mutations while working with Stadler, the pioneer of radiation effects in plants. A chromosome 2 recessive stock *lg gl b v₄* was pollinated by pollen from a dominant stock that had been treated with ultraviolet light. Most of the F_1 plants were dominant for the four genes studied. In a few cases, however, recessive types appeared in the F_1 plants. A cytological study of several virescent F_1 seedlings was made. Pachytene chromosomes revealed that about four fifths of the long arm of chromosome 2 was a single strand. In other words, there was a deficiency for most of the long arm of chromosome 2. The deficiency appeared terminal, as did a number of other ultraviolet-induced deficiencies, in contrast to most, or perhaps all, of the X-ray induced internal



Courtesy of Brookhaven National Laboratory

FIG. 16-14 Deficiencies in maize chromosomes induced by ultraviolet irradiation.

deficiencies. The ultraviolet-induced deficiency in chromosome 2, as well as several other chromosomes, is illustrated in Fig. 16-14.

DUPLICATIONS

Before salivary gland chromosomes in *Drosophila* were discovered, Sturtevant and Morgan (1923) made an extensive study of the Bar mutant in *Drosophila*. They came to the conclusion that it was not an ordinary mutant, but really a duplication of a small segment of the X chromosome. They decided this because Bar did not always breed true. In a small percentage of cases (one in one thousand six hundred) it produced normal flies as well as an extreme Bar, which was called Double Bar. They concluded that this condition was correlated with crossing-over.

After salivary gland chromosomes became available for analysis, Bridges (1936) demonstrated conclusively that the Bar "gene" was a duplication of a five-band segment in the X chromosome. The appearance of wild type (*B*) and Ultra Bar (*B B B*) among the offspring of a Bar female (*B B*) were shown to be caused by unequal crossing-over.

INVERSIONS

Another type of anomaly within a given chromosome is an inversion of a segment, i.e., rotation of a segment a full 180 degrees. If the normal order of genes is a b c d e f g h i j, in an inverted chromosome, we

might find the order to be *a b g f e d c h i j*, with the inverted segment shown in italics. In other words, the chromosome has been broken in two places, between b and c and also between g and h. In some instances of a double break, the broken piece might be inserted in the original position. This would be called restitution and would not be detected. The chromosome could appear normal.

If, however, the broken segment were rotated a full 180 degrees and re-joined to the two ends of the chromosome at b and h, the inversion could be detected cytologically. Suppose an inverted chromosome pairs with the normal homologue. At the first meiotic division (of the inversion heterozygote), the homologous regions are paired, giving rise to a typical "inversion configuration," a double loop. These inversions can have the centromere out-

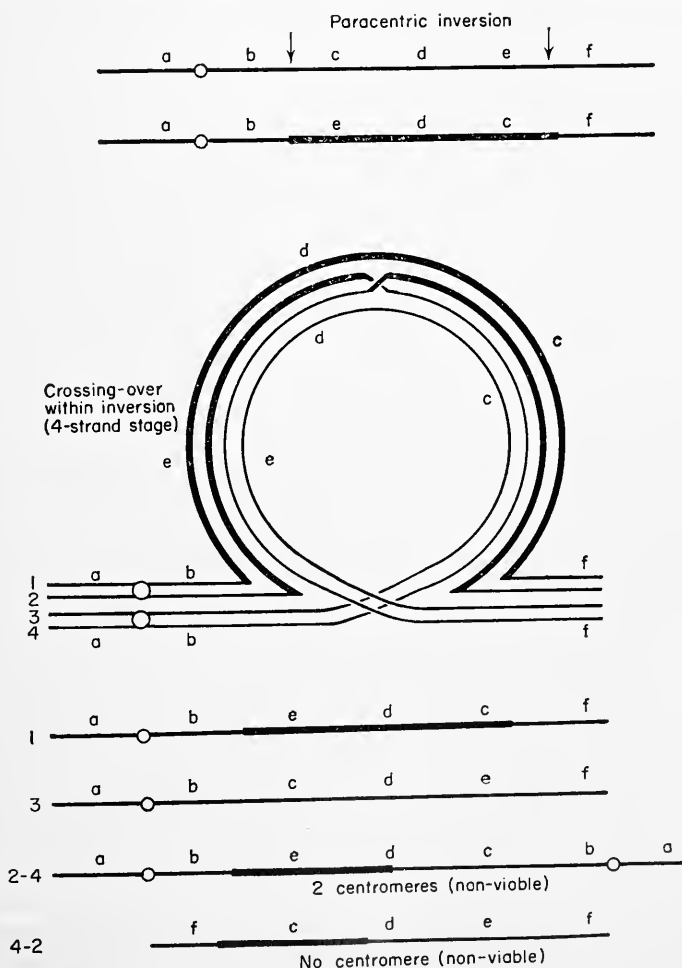


FIG. 16-15 Paracentric heterozygous inversion.

side the inverted segment (*paracentric*) illustrated by Fig. 16-15, or within the inverted segment (*pericentric*) as shown in Fig. 16-16. The types of chromatids formed by both paracentric and pericentric inversions are given in the illustrations, showing the consequences of crossing-over within the inverted segment.

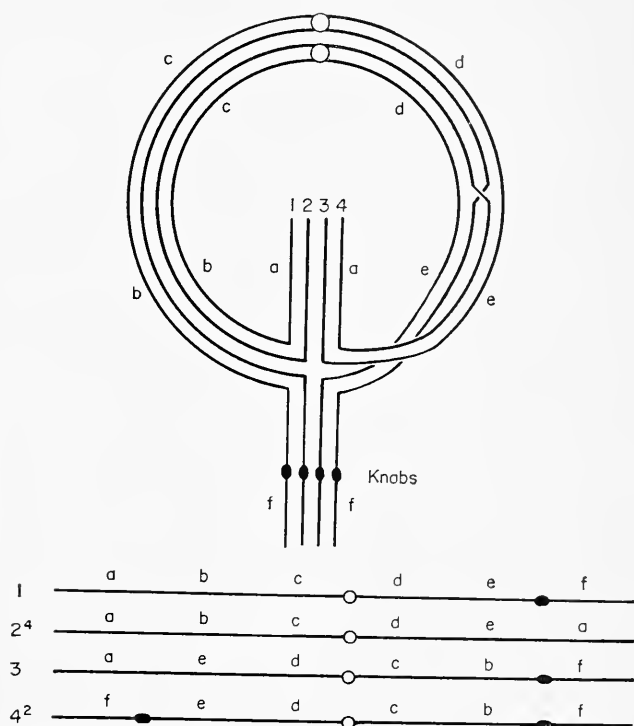


FIG. 16-16 Heterozygous pericentric inversion with centromere within inverted segment.

In paracentric inversions, such a crossover would result in two abnormal chromatids, one with two centromeres (*dicentric*) and one with none (*acentric*). Neither of these can undergo normal mitosis. Two noncrossover chromatids, one normal and one with an inverted segment, each with one centromere, are also formed. These two chromatids are the only ones functioning in the production of gametes. Consequently, an inversion effectively suppresses crossing-over. Geneticists have used this bit of information in planning experiments where it is desired to eliminate crossing-over in the inverted segment.

It is obvious that a crossover gives rise to nonviable gametes. In the case of a double crossover, the original 1:1 relationship of centromere to chromatid is restored, and the gametes are viable. Thus it is evident that an inversion *does not prevent crossing-over*, but merely renders chromatids with one

crossover nonviable. A chromatid resulting from a double crossover would produce a viable gamete, while one with three crossovers would be nonviable. These would be exceedingly rare, so only single and double crossovers need be considered. Even the double crossovers are rare compared to singles, so that inversion is a very effective inhibitor, as measured in the viable gametes.

If the centromere is within the inverted segment (pericentric), all the chromatids will possess one centromere, but crossovers will have an abnormal distribution of the portions of the chromosome. Consequently, most crossovers are nonviable, as they are for inversions in which the centromere is not within the inversion configuration, a paracentric inversion. This is shown diagrammatically in Fig. 16-16. The normal order of the genes was a *b c d e f*. The order in the inverted chromosome, however, was a *e d c b f*, with the centromere between *c* and *d* within the inversion. By tracing the chromatids in Fig. 16-16, you will note that the four chromatids will have the distribution of chromosomal segments found in Table 16-7. The centromere is indicated with a period.

Table 16-7. Chromatids Formed in Pericentric Inversion

(1)	a	b	c	.	d	e	f.....	normal chromatid
(2)	a	b	c	.	d	e	a.....	duplication a, deficiency f
(3)	a	e	d	.	c	b	f.....	inverted chromatid
(4)	f	e	d	.	c	b	f.....	duplication f, deficiency a

Chromatids 1 and 3 have all the portions of the chromosomes present, and can form viable gametes, but both number 2 and number 4 have a deficiency duplication situation which would in most cases fail to produce a viable gamete.

Hence the end results are the same, whether the centromere is within the inverted segment or on the outside of it. The chromatids produced by a single crossover in the loop of an inversion heterozygote are not viable and the inversion is a most effective suppressor of crossing-over.

GENETIC CONSEQUENCES OF INVERSIONS

Inversions can be detected genetically as well as cytologically. In fact they were first detected in *Drosophila* through a changed order in linkage groups long before their cytology was known (Sturtevant, 1921). If the normal order of genes is a *b c d e f*, and in the inverted chromosome a *e d c b f*, it is apparent that gene *b* will show much closer linkage to *f* than in the normal chromosome. The failure to recover viable crossover gametes in an inversion heterozygote makes genetic tests somewhat laborious. For a thorough discussion of both cytological and genetic consequences of inversions the reader is referred to C. P. Swanson, *Cytology and Cytogenetics* (1957).

It is possible to produce an inversion homozygote in which two homologous chromosomes have their regions inverted. In this case pairing would be perfectly normal, with complete fertility, but with a new linkage order established.

Suppose, for example, an inversion is obtained in the short arm of chromosome 9 in maize. The normal order of genes, with approximate crossover values, is shown in Table 16-8.

Table 16-8. Linkage of Genes in Chromosome 9 with Crossover Values Listed Between Genes

yg_2	yellow green seedling
7	
C.....	aleurone color
3	
sh	shrunken endosperm
2	
bz	bronze anthocyanin
13	
bp	brown pericarp
15	
wx	waxy starch

If the breaks to form the inversion are between yg_2 and C , and also between bp and wx , the inverted chromosome would then have its genes in the order $yg_2 bp bz shC wx$. It could be made homozygous and linkage tested for those genes in the short arm of chromosome 9. In such a stock the yellow green seedling yg_2 would show rather loose linkage with C , but rather close linkage to brown pericarp bp . In the original chromosome the crossover value between yg_2 and C is seven units; and the value for yg_2 and bp is more than 30 units.

TRANSLOCATIONS

The last type of a chromosomal anomaly to be discussed is that of a translocation, sometimes referred to as a reciprocal translocation or segmental interchange. If two nonhomologous chromosomes are broken and the parts are rejoined to the wrong partners, then we have a situation wherein all the gene loci are present, but in an abnormal distribution.

Historically, we have had translocations with us since the early 1920's. The

first case to be thoroughly analyzed was found in the evening primrose *Oenothera*, the plant studied by De Vries in working out his mutation theory. He was impressed with a few unusual types that occurred in an otherwise fairly uniform population, and he considered these to be mutations. From this beginning he developed the mutation theory of the evolution of new species. (This subject will be discussed fully in Chapter 21.) It so happened that these "mutations" in *Oenothera* were not typical gene mutations, but rather chromosomal abnormalities resulting from the dissociation of the chromosomes of the "normal *Oenothera* complement."

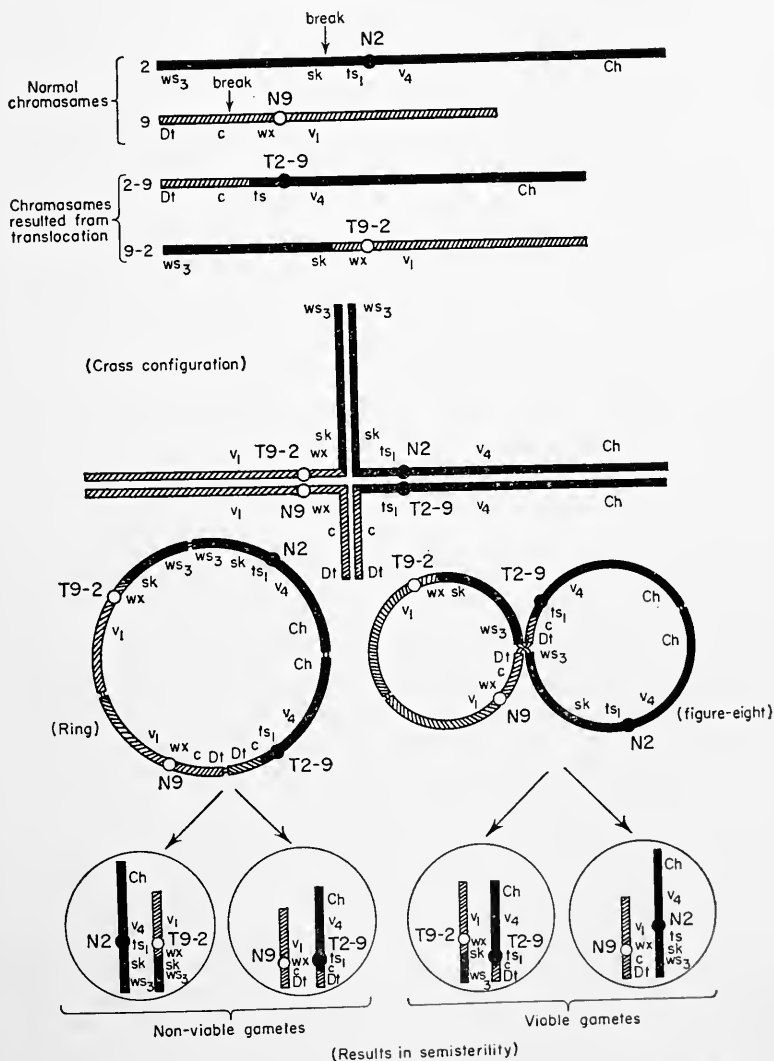


FIG. 16-17 Diagram of a translocation figure (T 2-9 in maize).

This normal *Oenothera* complement is quite an exception to most of the plant kingdom. Ralph E. Cleland was the one who solved the mystery of the peculiar cytological and genetic behavior of *Oenothera*, and presented a logical explanation of the mutations of De Vries. *Oenothera* has 14 somatic chromosomes, the same number as the peas used by Mendel. Unlike peas, however, the 14 chromosomes of *Oenothera* do not form seven pairs at meiosis, but rather they form one continuous ring of 14 chromosomes, joined end to end.

Why are they joined end to end? According to Cleland (1949), it was John Belling, noted for his cytological work especially with *Datura*, who advanced the explanation. He suggested that a ring of four chromosomes in *Datura* took this particular configuration because two nonhomologous chromosomes had exchanged, or traded a portion of their chromosomes. This is illustrated in Fig. 16-17. Since homologous parts of the chromosomes pair, it is evident that a plant (or animal) with a translocation would present a crosslike figure at prophase, when the chromosomes are tightly paired. At diakinesis the ends of the chromosomes are still joined, but the rest of the chromosomes are not closely paired, so that the "cross configuration" becomes a ring of four, if a single translocation is involved.

In *Oenothera*, the circle of 14 chromosomes is the result of a series of successive translocations. To use Cleland's illustration, each chromosome could be numbered by two numerals connected by a dot. One set of 14 chromosomes might be known as 1.2, 3.4, 5.6, 7.8, 9.10, 11.12, and 13.14. The other set could be labeled 2.3, 4.5, 6.7, 8.9, 10.11, 12.13, and 14.1. A plant having these two sets of chromosomes would form a ring of 14. Many other combinations can also produce this effect.

The number of chromosomes involved in a ring (or rings) can vary from none (7 pairs) to the ring of 14 (0 pairs). Actually, in the genus *Oenothera*, these two possibilities exist, as well as numerous other combinations of rings and pairs.

GENETIC CONSEQUENCE OF TRANSLOCATIONS IN OENOTHERA

Since all 14 chromosomes are coupled together in a large ring, and since adjacent chromosomes go to opposite poles at meiosis, it is evident that all characters in *Oenothera* would behave as though in one linkage group. Gametes produced are identical with those that united to form the plant, since paternal and maternal chromosomes alternate in the ring, and since adjacent chromosomes go to opposite poles. In rare instances this regular scheme is not followed exactly. Such irregularities, and many kinds occur, result in mutations.

TRANSLOCATION IN MAIZE

This was first observed because of a semi-sterile character associated with the maize translocation. This semi-sterile condition can be observed in the pollen, where approximately one half of the pollen grains are well filled with starch. The other half appears empty and shriveled.

The most noticeable effect is a semi-sterile ear, one which has only approximately half as many kernels as a normal, well-filled ear (Fig. 16-18). This is



FIG. 16-18 Semi-sterile ear of corn resulting from a translocation (bottom), and a normal ear of corn, showing only about 50% kernels on ear in comparison with normal. The pollen is also 50% sterile.

brought about by the failure of approximately one half of the female gametes. Normally the kernels in the separate rows on the ear are rather tightly packed together, with the individual kernels being flattened because of the pressure of adjacent kernels. When one half of the gametes are lethal, each kernel has more room to develop. This results in the individual kernel's being rounded. Phenotypically, a semi-sterile ear is quite distinct from a normal ear. The difference led investigators to examine the pollen, which was shown to have approximately half of the grains poorly filled with starch, in contrast to the normal round pollen grains.

A cytological examination of the chromosomes at pachytene revealed the cause of the failure of all gametes to develop. It was found that instead of having ten pairs of chromosomes at meiosis, there were but eight pairs, with

the other two pairs forming a ring similar to that described for *Datura* and *Oenothera*. The ring is formed at diakinesis. It follows a cross-shaped configuration at pachytene, brought about by the pairing of homologous parts of the two pairs of chromosomes. The pairing takes place up to the point where the interchange of chromosomal segments occurred (Fig. 16-17).

Half of the gametes containing chromosomes from this ring are viable, the other half nonviable. If the two normal maize chromosomes go into one gamete, the gamete will be viable because all of the genes of the two chromosomes are present. Likewise, if the two new translocated chromosomes enter a gamete, the gamete will be viable and functional. This is because all of the

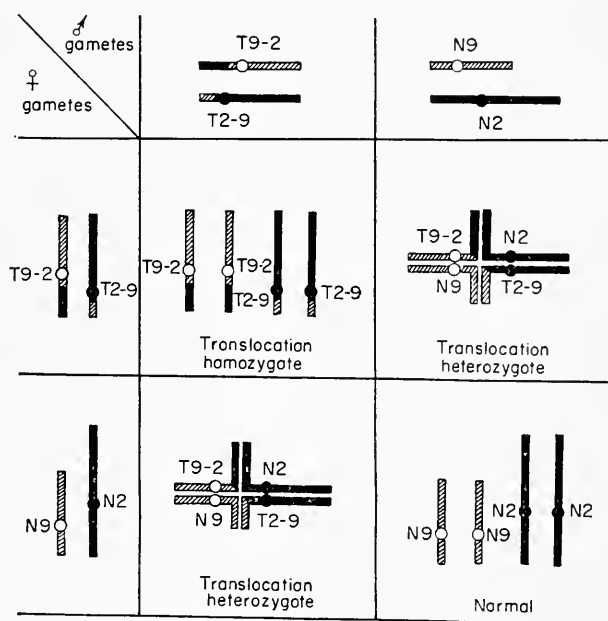


FIG. 16-19 Zygotes produced by viable gametes in a heterozygous translocation.

genes of the two chromosomes are present, even in the two new synthetic chromosomes.

If either normal chromosome should find its way into a gamete with a translocated one, the gamete will be nonviable because it will be deficient for a portion of one chromosome and will have a duplication for a portion of the other. These are the four types of gametes formed, since the centromeres of the individual chromosomes go to opposite poles in meiosis. From a ring of four chromosomes, those opposite with different centromeres must go to the same pole together at the reduction division, in order to form viable gametes. This is accomplished by a twist of the ring into a figure 8 at metaphase, presenting nonhomologous centromeres toward the same poles (Fig. 16-17). Zygotes from viable gametes are shown in Fig. 16-19.

NEW SYNTHETIC CHROMOSOMES RESULTING FROM TRANSLOCATIONS

It is possible to produce entirely new chromosomes with a new linkage of genes as a result of translocations. Normally, each chromosome contains genes that are linked to each other but show independent assortment with other linkage groups.

With radiation, it is possible to produce breaks in the chromosomes and to obtain translocations with ease. Suppose we produce a translocation between chromosomes 2 and 9 in maize, with the break between *wx* and the centromere in chromosome 9 and with the break between *ws₃* and *lg* in chromosome 2. (See Fig. 8-10 for relative position of these genes.) We would then have the genes in the short arm of chromosome 9 linked with most of the genes in chromosome 2, as shown in Fig. 16-20. The translocation can be made homozygous by selfing heterozygous plants. The only two kinds of viable gametes are (a) those containing a normal chromosome 2 with a normal 9, and (b) those containing two translocated 2 and 9 chromosomes.

The zygotes obtained can be found in a conventional checkerboard similar to Fig. 16-19. The zygote in the upper left hand corner represents a new type, with all of the genes of the former chromosomes 2 and 9 present, but in a new "linkage group." Most of the known genes of chromosomes 2 and 9 are now combined in one large linkage group, with the remaining genes in a different group. Since all genes are present, although in new groups, and since each chromosome has a homologous partner, the pairing at meiosis is normal and complete fertility is restored. This compares with approximately 50% sterility for the translocation heterozygote. An ear of a new translocation homozygote is indistinguishable phenotypically from a normal ear of corn with no translocation. If, however, the homozygous T 2-9 translocation is crossed with any normal corn, the F₁ hybrids *all* will be heterozygous for the translocation and have the sterility characteristic of the translocation heterozygotes. They will show the typical cross configuration at pachytene.

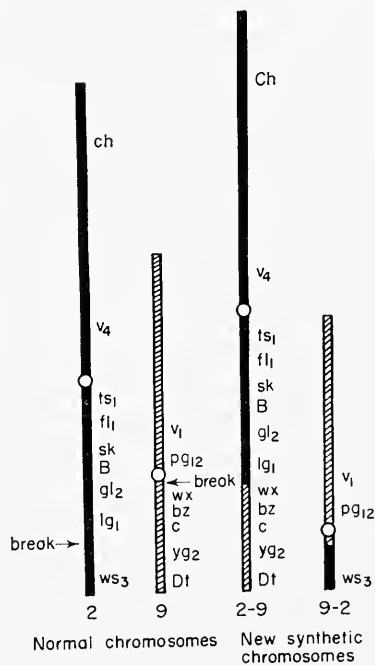


FIG. 16-20 Translocation of chromosomes 2 and 9 in maize results in two new chromosomes with new linkage relationships.

Thus it is possible in the laboratory to change the architecture of existing

chromosomes, while at the same time rearranging linkage groups into new ones, an exciting application of the knowledge of genetics and cytology.

TRANSLOCATIONS AS GENETIC MARKERS

Translocations serve as admirable genetic markers when the translocation heterozygote produces a phenotypic effect. In maize the ear of the heterozygote has only about 50% of the normal number of kernels (Fig. 16-18). The heterozygote can also be identified by an examination of the pollen, which has about half of the grains round and well filled with starch, while the remaining half are empty.

Let us return to the translocation T 2-9 discussed in the previous section, and whose chromosomes are pictured in Fig. 16-17. In this plant let us assume that the recessive genes were each contributed by the translocated chromosomes, with the dominant alleles contributed by the normal. The order of the linked genes in the new synthetic chromosomes would then be $ws_3 \cdot v_1$ and $dt\ yg_2\ c\ sh\ bz\ bp\ wx \cdot lg\ gl_2\ B\ sk\ v_4$ with the dominant alleles in the normal chromosomes 2 and 9. If an F_1 heterozygous plant is testcrossed to a plant with normal chromosomes and homozygous for all recessive genes, it will be possible to establish crossover values between the different genes and the point of breakage. The point of breakage (T) will appear phenotypically as a semisterile ear and 50% empty pollen. We may let t represent the phenotype of a normal ear.

Suppose we study two characters, one close to the point of breakage, wx , and one far removed, v_4 . Comparing the waxy-starchy segregation and the semisterile (T) versus normal ears (t), there will be four classes of gametes produced by the F_1 . The results of a testcross are shown in Table 16-9.

Table 16-9. Testcross of Heterozygous T 2-9 Translocation, Showing Linkage of wx and T

Class	Gamete	Testcross Progeny	Phenotype
(1) Parental (linkage)	$wx\ T$	$wx\ T/wx\ t$	waxy, semisterile
(2) Parental (linkage)	$+ t$	$+ t/wx\ t$	nonwaxy, normal
(3) Crossover	$wx\ t$	$wx\ t/wx\ t$	waxy normal
(4) Crossover	$+ T$	$+ T/wx\ t$	nonwaxy, semisterile

Classes 1 and 2 represent the parental or linkage group, while 3 and 4 represent crossing-over between the point of breakage and the waxy locus. We would expect to find classes 1 and 2 in great excess over 3 and 4, since the wx locus is close to the point of translocation (T) and classes 3 and 4 would occur only when there was a crossover between the wx locus and the point of translocation.

A comparison of the results with the $v_4/+$ segregation would also produce four classes as shown in Table 16-10.

Table 16-10. Testcross of Heterozygous Translocation T 2-9,
Showing Linkage of v_4 and T

Class	Gamete	Testcross Progeny	Phenotype
(1) Parental (linkage)	$v_4 T$	$v_4 T/v_4 t$	virescent, semisterile
(2) Parental (linkage)	$+ t$	$+ t/v_4 t$	green, normal
(3) Crossover	$v_4 t$	$v_4 t/v_4 t$	virescent, normal
(4) Crossover	$+ T$	$+ T/v_4 t$	green, semisterile

In the latter case we would not expect to find classes 1 and 2 so much in excess (as was the case for wx and the translocation point), since v_4 is located a considerable distance from the point of breakage. There is ample opportunity for crossing-over in the region between v_4 and T.

Thus we see that translocations can serve as effective genetic markers, since they produce a distinctive phenotype. They are useful in testing new genes that have not as yet been located in one of the known linkage groups (ten for maize). Not only that, but indications may be obtained simultaneously for two linkage groups, for two nonhomologous chromosomes are always involved in a translocation. Since translocations can be produced by mutagens in great abundance, man can create his own genetic markers for the different linkage groups. In maize the different translocations can be identified with certainty, both genetically and cytologically.

INVERSIONS AND TRANSLOCATIONS IN DROSOPHILA AND SCIARA

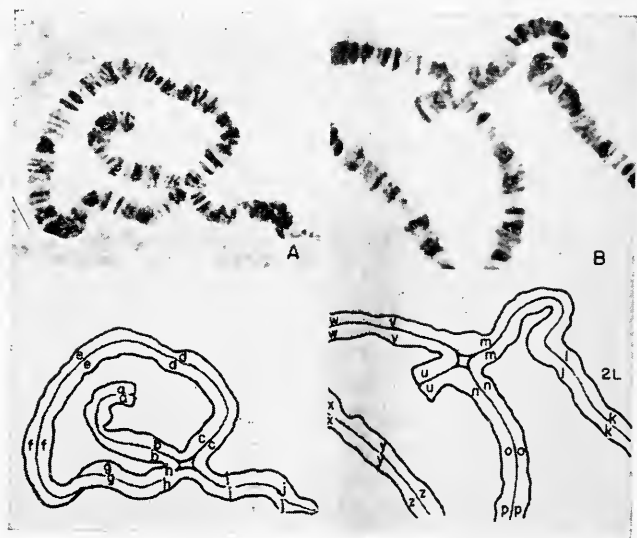
In both *Drosophila* and *Sciara*, two genera in the order Diptera, the study of salivary gland chromosomes has enabled investigators to detect cytologically, with great precision, both inversions and translocations. Since the bands on the chromosomes are known to be associated with the location of known genes, it is possible to obtain precise information on both the genetics and cytology of inversions and translocations. The behavior outlined for maize inversions and translocations is applicable to *Drosophila* and *Sciara*.

Excellent cytological preparations of the salivary gland chromosomes showing inversions and translocations are shown in Figs. 16-21 and 16-22.

CONCLUDING REMARKS

The normal chromosome constitution of higher plants and animals is the diploid condition with n pairs of chromosomes, each chromosome

having a homologous partner or mate. The pairs are evident at meiosis. In plants, different species of a genus may be arranged in a polyploid series, with numbers representing species multiples of a basic number. For example, wheat species have 7, 14, and 21 pairs of chromosomes.

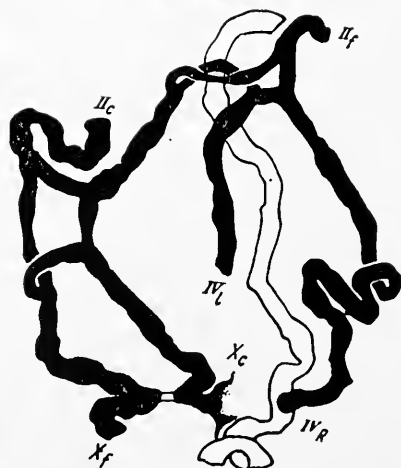
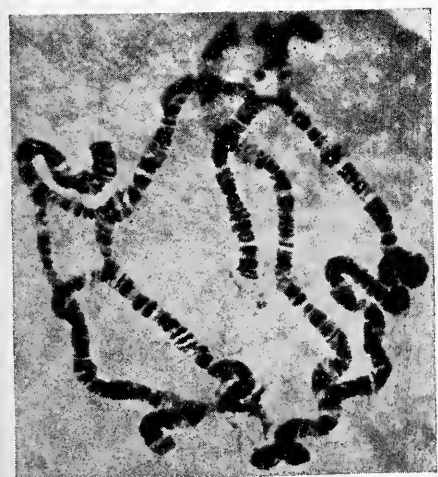


Courtesy of Demerec and Kaufmann;
and the Carnegie Institution of Washington

FIG. 16-21 Heterozygous inversion and translocation in *Drosophila*. The drawings show points of breakage in photomicrographs. (A) Pairing between two X chromosomes, one of which contains an inverted section. In the diagram below (A), the chromosome carrying the wild-type sequence of genes has been labeled *abcdefghi*, and the chromosome bearing the inverted section has the sequence *abghfedcij*. (B) Pairing between two normal chromosomes and two that have exchanged sections. The second and third chromosomes are involved (2L and 3R). In the diagram below, 2L bears the letters *klmnop* and 3R the letters *uvwxyz*. The two chromosomes that have exchanged parts carry the sequences *klmvwxyz* and *unop*. Pairing between these chromosomes and those carrying the unaltered sequence of parts produces the cross-shaped configuration.

The chapter is concerned with variations from the normal condition of a certain number of pairs of chromosomes. The architecture of the chromosomes may be changed by the addition or subtraction of whole sets (genomes) of chromosomes and of individual chromosomes. Also the architecture of an individual chromosome may be changed by the addition, subtraction, or rearrangement of the gene loci, or the genes of two or more different chromosomes may be rearranged as in translocations. Any of these rearrangements of genes may give rise to certain hereditary manifestations.

It is through a study of the exceptional cases discussed in this chapter that our understanding of genetics and cytology has increased markedly. The sep-



Courtesy of Helen V. Crouse; and Chromosoma

FIG. 16-22 (Above) Heterozygous inversion in *Sciara coprophila*. (Below) Female *Sciara* heterozygous for two translocations.

arate sciences of genetics and cytology have been united in the combined science of cytogenetics.

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PROBLEMS

16-1. Define or describe the following terms:

acentric	heterochromatin
aneuploid	hexaploid
autopolyploidy	inversion
autotetraploid	mid-prophase
centromere	monoploid
chromocenter	nullisomic
chromosomal balance	pachytene
colchicine	paracentric inversion
deficiency (deletion)	pericentric inversion
dicentric	salivary gland chromosome
diploid	semisterile maize
disomic inheritance	syndrome
duplication	tetraploid
euchromatin	tetrasomic
euploid	translocation
genome	trisomic
haploid	trisomic inheritance

16-2. Identify the following scientists, giving a major contribution with an approximate date.

Belling, John	McClintock, Barbara
Blakeslee, A. F.	Morgan, T. H.
Bridges, C. B.	Painter, T. S.
Cleland, R. E.	Sax, K.
Dobzhansky, Th.	Sears, E. R.
Eigsti, O. J.	Stadler, L. J.
Kaufmann, B. P.	Sturtevant, A. H.

16-3. It is often said that an inversion suppresses crossing-over. Is this literally true? If not, what is meant? Illustrate with a diagram.

16-4. How may induced deficiencies be used in locating genes at specific locations in the chromosomes? (Maize may be used as an example.)

16-5. Show by a diagram how a translocation behaves in meiosis in maize. Assume a translocation between chromosomes 8 and 9. (Any two chromosomes could be involved.) What is the phenotypic expression of the ear? Show which gametes are viable; also show what zygotes would be produced from viable gametes.

16-6. Translocations serve as useful genetic markers particularly in maize because the translocation produces a distinct phenotypic effect, a semi-sterile ear or 50 per cent empty pollen.

In an experiment of C. R. Burnham, involving genes in chromosome 9 (plus an 8—9 translocation) the following phenotypes were recovered in a testcross. *T* is the conventional symbol for translocation, and *t* for normal (no translocation).

+	wx	T	205	(plump, waxy starch, semi-sterile ear)
sh	+	t	171	(shrunken, normal starch, normal ear)
+	+	t	82	(plump, normal starch, normal ear)
sh	wx	T	49	(shrunken, waxy starch, semi-sterile ear)
+	wx	t	40	(plump, waxy starch, normal ear)
sh	+	T	17	(shrunken, normal starch, semi-sterile ear)
+	+	T	6	(plump, normal starch, semi-sterile ear)
sh	wx	t	3	(shrunken, waxy starch, normal ear)

The genetic markers are in the correct order. Calculate the crossing-over for regions 1, 2, 1 and 2, and the coefficient of coincidence.

- 16-7. Suppose a corn plant trisomic for chromosome 4 and homozygous for the *Su* gene (*i.e.* *Su/Su/Su*) were crossed by an *su* stock. A plant of 21 chromosomes was then selected for further study. If pollen from this plant were applied to an *su/su* plant, what ratio of *Su* to *su* would be obtained? (Remember $n + 1$ pollen grains rarely function.)
- 16-8. If the pollen from the trisomic *Su/Su/su* plant were applied to a normal *Su/su* plant, what ratio of *Su* to *su* kernels would be expected?
- 16-9. The same plant used in Problem 16.8 was heterozygous for the *Y/y* gene located in chromosome 6. What ratio of *Y* to *y* kernels would be obtained if this plant were self-pollinated?
- 16-10. In maize it is possible to produce a plant that is a homozygous translocation stock. This is produced when two gametes with translocated chromosomes unite. Such a homozygous translocation stock is completely fertile, since the chromosomes (even the new translocated ones) pair completely. The pollen also is fertile. Phenotypically the new stock is indistinguishable from a normal fertile strain with no translocation. If you were given two ears, one a normal and the other a homozygous translocation, how would you determine which was which? What results would be expected from the crosses you would make?

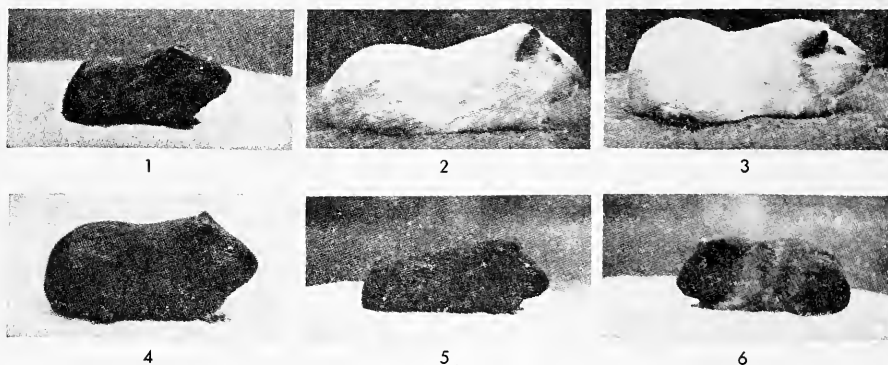
Induced Mutations—Experimental Alteration of the Germ Plasm

THE TITLE chosen for this chapter, "Induced Mutations—Experimental Alteration of the Germ Plasm," seems more appropriate than the term *radiation genetics*, sometimes used to describe the phenomenon of induced mutations. Actually, many chemicals now are known to be mutagenic. These would be excluded if we limited the mutagens to different kinds of radiation.

HISTORICAL NOTES

Scientists have been interested for a long time in experimental modification of the germ plasm, in fact, almost as long as we have been aware that it is a separate entity from the somatic cells. Weismann's classic work on the germ plasm was published (1892) just three years before the discovery of the X rays by William Roentgen in 1895. It was Roentgen's discovery, and subsequent researches, that have done so much to change the germ plasm, which once seemed constant and not subject to alteration by environmental conditions. At the time Weismann proposed the germ plasm theory, there was a controversy of considerable proportions as to whether characters acquired by an individual could be transmitted to its progeny. Weismann was convinced that acquired characters could not be transmitted, and demonstrated his point by cutting off the tails of mice for several generations. The progenies always were born with tails. The tails of lambs had been docked for centuries without producing a short-tailed sheep.

Weismann's experiment was essentially a negative one. It lacked the sophistication of a positive experiment conducted at Harvard University by Castle and one of his colleagues, John C. Phillips, during the first decade of

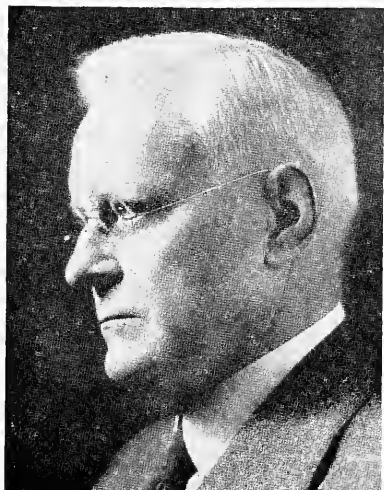


Courtesy of W. E. Castle; and Genetics and Eugenics, Harvard University Press

FIG. 17-1 Ovaries of black guinea pig (1) transplanted into albino female (2) whose ovaries had been removed. Mating of albino ♀ (2) to an albino ♂ (3) produced all black young (4, 5, and 6).

this century. They removed the ovaries of an albino guinea pig, and sometime later replaced them with the ovaries of a young black female.

Later the albino female was mated to an albino male and produced three litters of guinea pigs, all black, showing that the cellular environment of the ovary has no influence on the germ cells within (Castle and Phillips, 1911). This classic experiment really dealt a mortal blow to the early theory of the inheritance of acquired characters (Fig. 17-1).



Courtesy of W. J. Robbins; and the Torrey Botanical Club

FIG. 17-2 C. Stuart Gager, a pioneer in the use of radiation on plants.

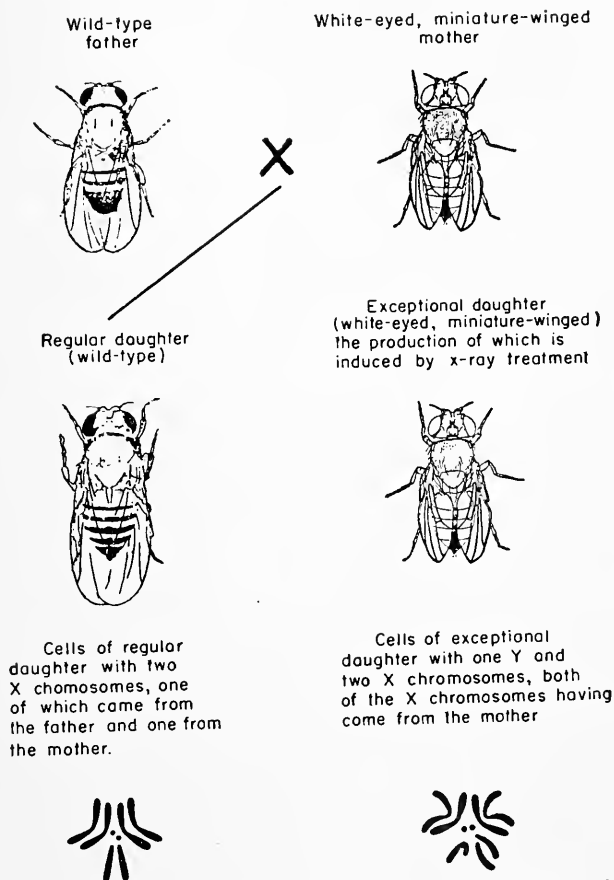
GAGER'S RADIATION EXPERIMENTS WITH PLANTS

Less than ten years after the discovery of X rays by Roentgen in 1895, investigators were using X rays and similar radiation from radium, in attempts to alter the germ plasm of plants. One of the first was C. Stuart Gager at the New York Botanical Garden. Between 1905 and 1910 he published ten papers concerning the effects of radiation on plants. Physiological effects, all injurious, were noted in a number of cases.

MAVOR'S RADIATION EXPERIMENTS

During the 1920's, James W. Mavor, at Union College in Schenectady, conducted a number of interesting experiments, in which he used

X rays on *Drosophila*. From 1921 to 1929, he published 20 papers on radiation effects. He clearly demonstrated a genetic effect that was the result of a nondisjunction, in which a few of the offspring received two X chromosomes from the mother, plus a Y from the father. These flies were phenotypic females, since the ratio of X chromosomes to autosomes was 1:1. White-eyed,



Courtesy of J. W. Mavor; and Scientific Monthly

FIG. 17-3 White-eyed daughter produced by mating $w \text{ } \varnothing \times + \text{ } \delta$ in *Drosophila*. Exceptional fly had two X chromosomes (from mother) and one Y chromosome.

miniature-winged females were treated with X rays and mated to a wild male. The results are shown in Fig. 17-3, taken from Mavor's 1925 paper on the "Attack on the Gene." This represents a clear-cut case of a genetic effect induced by X rays. It is true that the immediate effect was chromosomal, but so were many of the gene losses later studied more extensively.

MULLER'S RADIATION EXPERIMENTS

The radiation experiments of Muller demonstrated beyond doubt that the mutation rate following radiation with X rays was several times the spontaneous rate. His paper, "The Artificial Transmutation of the Gene," is a classic of genetic literature. The significance of his work was not that he was the first to use X rays to produce mutations (which he was not). Rather, it was his clever experimental approach, which established unequivocally a marked increase over the spontaneous mutation rate. These methods will be described shortly. A recent photograph of Muller is shown in Fig. 17-4.



FIG. 17-4 H. J. Muller, pioneer in use of radiation to induce gene changes.

METHODS AND TECHNIQUES OF DETECTING INDUCED MUTATIONS—THE *CIB* METHOD

This method was devised by Muller to detect *all* of the mutations in the X chromosome of *Drosophila*. Although used primarily to study radiation-induced mutations, it works equally well in the study of spontaneous mutants or those induced by other mutagens, such as many chemicals.

The *C* of the *CIB* stands for a long inversion that effectively prevents crossing-over within the inverted segment of the chromosome. Crossing-over can still occur, but gametes containing crossovers rarely survive, so it is an effective crossover suppressor, as was pointed out in Chapter 16. The *l* designates a recessive lethal, causing homozygous *l/l* females and hemizygous males *l/Y* to be nonviable. The *B* stands for the Bar eye, which is used as a marker for the flies desired to mate in the X_1 generation.

The operation of this method is shown in Fig. 17-5. It is extremely simple. A *CIB* ♀ is mated to a male that has been irradiated. The purpose is to detect all lethal mutations that have been induced in the X chromosome of the irradiated male. Success is based on the fact that the Y chromosome is essentially inert, so that a male is hemizygous for all genes in the X chromosome. Hence if a male can be treated, and the X chromosome can be followed through into a succeeding generation until it becomes the X chromosome of a new male fly, then the presence of any gene for lethal can be detected by the absence of males in the population.

The first generation resulting from the sperm of the X-rayed male is conventionally known as the X_1 generation, since X rays were used as the muta-

gen. Sometimes this generation is known as the R_1 generation (for radiation) or the M_1 generation if a chemical mutagen is used.

In the X_2 resulting from this mating, all males with the *CIB* X chromosome die because of the lethal gene *l*, which is unprotected by the inert Y chromosome. Likewise, if a gene for lethal zygote has been induced in the X chromosome of the original treated male, all males in the X_2 carrying the treated X

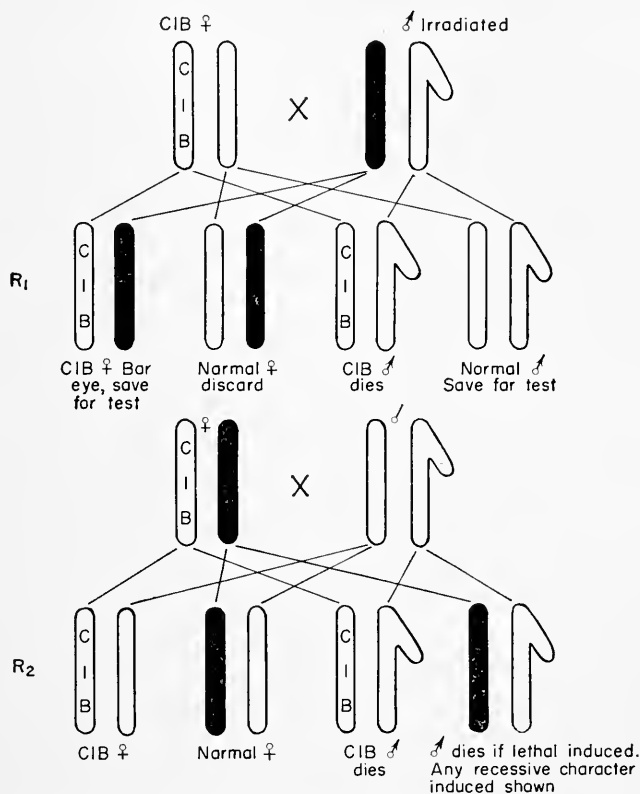


FIG. 17-5 Muller's *CIB* method for detecting mutant genes in the X chromosome of *Drosophila*

chromosome also die. Thus the X_2 progeny would consist entirely of females. In carrying out this test, it is necessary that only *one* female fly be tested in each X_1 culture. Thus it is possible to trace all the X_2 progeny to a single treated X chromosome. A few of the X_2 culture bottles may contain *only females*. These constitute the evidence for the induction of a lethal mutation in the original treated chromosome. Each normal female (not *CIB*) carries the induced lethal in the heterozygous condition for further study. Any visible mutation would appear, of course, among the surviving males in the X_2 culture.

THE MULLER-5 METHOD

The *CIB* method has largely been superseded by the *Muller-5 method* for detecting lethals in the X chromosome (Fig. 17-6). The Muller-5 female is genetically marked by apricot eye w^a/w^a and Bar eye (B/B), in addition to a long inversion to eliminate crossing-over.

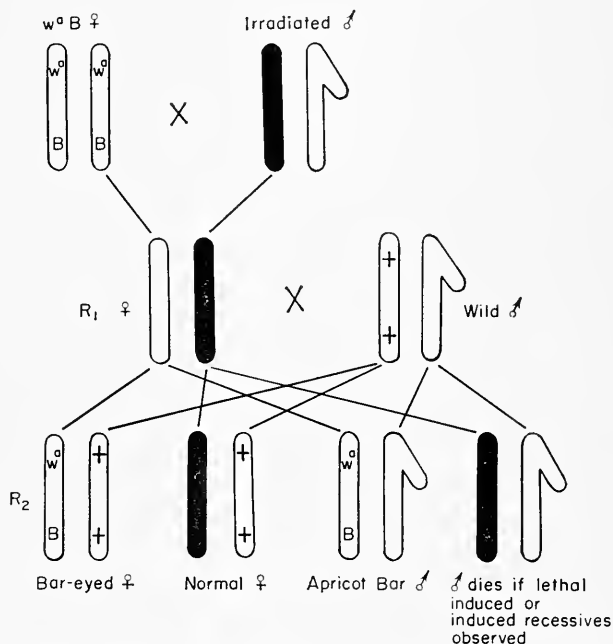


FIG. 17-6 Muller-5 method for detecting mutant genes in X chromosome in *Drosophila*.

A homozygous apricot Bar-eyed female is mated to a male that has been X-rayed, or treated in some way to induce mutations. The X_1 female, which is Bar-eyed, is mated to a wild male. She has two kinds of X chromosomes, the $w^a C B$ and the treated one. The treated X will become hemizygous (unprotected) in the males resulting from this mating, and will reveal any lethals or any other recessive genes that were masked in the X_1 fly. In most progenies one half of the males are apricot-Bar, the other half normal. However, in a small percentage of cases where a lethal has been induced, *all* of the males will be apricot-Bar. In case a recessive mutant for a morphological character has been induced, one half of the males would be of this phenotype, the other half apricot-Bar.

The success of this method, like that of the *CIB*, depends upon testing the

X_1 female flies *individually*, so that one half of the progeny can be traced directly back to a treated chromosome.

These methods, while simple in operation, represent considerable ingenuity in their original concept. Muller demonstrated unequivocally a marked increase in the mutation rate in *Drosophila*. For these original researches and many that followed, he was awarded the Nobel Prize in Medicine in 1946.

BALANCED LETHALS FOR DETECTING INDUCED MUTATIONS IN THE AUTOSOMES

The balanced lethal, the Curly Lobe Plum system discussed in Chapter 12, has been used effectively in detecting lethal mutants in chromosome 2 in *Drosophila*. This method is similar to those described for the X chromosome, but somewhat more complicated because it is necessary to induce a mutant in a chromosome and then secure flies that are homozygous for this one treated. Every treated fly has two No. 2 chromosomes. If we label these 2A and 2B, we can see that it becomes necessary to make a fly (either male or female) homozygous 2A/2A or 2B/2B to detect any induced recessive mutants. Fortunately, this can be done with the Curly Lobe Plum balanced lethal system. A $Cy+L/+Pm+$ female fly is mated to an X-rayed wild male with two identical No. 2 chromosomes, which we shall label 2A and 2B. The mode of operation is shown in Fig. 17-7.

Four kinds of X_1 result from this mating, as shown in the diagram. Two are Curly Lobe and two are Plum, which have treated chromosomes either 2A or 2B. *One fly is selected* and back-crossed to Curly Lobe Plum. Four kinds of flies will result from this mating, as indicated in the diagram. There will be a $Cy+L/Cy+L$ (lethal), a $Cy+L/+Pm+$, a $Cy+L/2A$, and a $+Pm+/2A$. In the latter two individuals, only one treated No. 2 chromosome is present, depending upon which fly was selected to make the backcross. It is either 2A or 2B, and cannot possibly be both types.

From this backcross progeny again *one* type is selected, either Curly Lobe or Plum, and intercrossed. Suppose a Curly Lobe fly is selected. The homozygous $Cy\ L/Cy\ L$ flies die, leaving two classes, two thirds of which are Curly-Lobe and one third normal, provided no mutants have been induced. If a lethal had been induced, there would be only Curly Lobe flies in the progeny. If a recessive visible mutation were induced, one third of the progeny would be of this type, as represented by the 2A/2A type. If a subvital or semilethal mutant had been induced, then the number of Curly Lobe flies would be $> \frac{2}{3}$ and the number of normals $< \frac{1}{3}$ approaching zero, as the deleterious nature of the mutant increased.

The important feature of this test is that it is a device for rendering an individual chromosome homozygous, so that *all* recessive mutants in that chromosome can be detected.

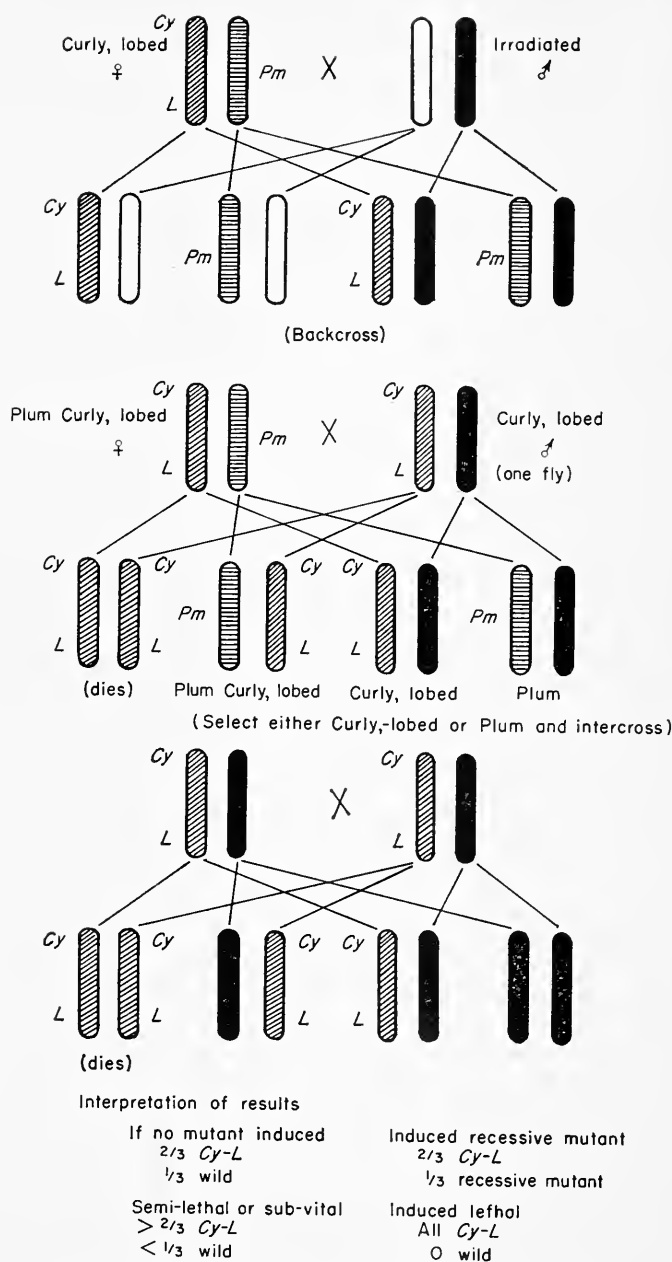


FIG. 17-7 Curly Lobe Plum method for detecting mutant genes in chromosome 2 in *Drosophila*.

STADLER'S RADIATION EXPERIMENTS

Another investigator who worked at the same time as Muller and who made an outstanding contribution to induced mutations by radiation was Stadler at the University of Missouri. His experimental materials were barley and maize. He found marked increases over the spontaneous mutation rate in both of these cereals. In maize he established with considerable precision the spontaneous mutation rate for a number of endosperm characters. He used the crossing plot technique proposed by Shull for producing hybrid seed. A photograph of Stadler, along with Shull and with Jones of hybrid corn fame, is found in Chapter 12, Fig. 12-8.

METHOD FOR DETECTING ENDOSPERM MUTATIONS IN MAIZE

Stadler (1942) made an intensive study of the spontaneous mutation rate in maize, using the crossing plot method of the commercial hybrid seed planters. A genetic stock dominant for a number of genes was grown as the female parent, and the tassels were all removed before any pollen-shedding. These plants received pollen from a multiple recessive stock grown in about every fifth row to supply pollen for the detasseled rows. In this way Stadler was able to observe large populations (numbering in the millions). This is a requisite for a spontaneous mutation study, since the rate of spontaneous mutation is rather low. His results are shown in Table 17-1, taken from his work.

Table 17-1. Spontaneous Mutation Rate Found by Stadler in ♀ Gametes for Eight Specific Genes in Maize

Gene	No. ♀ Gametes	No. of Mutations	Frequency per 10^6 Gametes
<i>R</i>	554,786	273 (<i>r</i>)	492
<i>Ci</i>	265,391	28 (<i>ci</i>)	106
<i>Pr</i>	647,102	7 (<i>pr</i>)	11
<i>Su</i>	1,678,736	4 (<i>su</i>)	2
<i>Ci</i>	426,923	1 (<i>c</i>)	2
<i>Y</i>	1,745,280	4 (<i>y</i>)	2
<i>Sh</i>	2,469,285	3 (<i>sh</i>)	1
<i>Wx</i>	1,503,744	0 (<i>wx</i>)	0

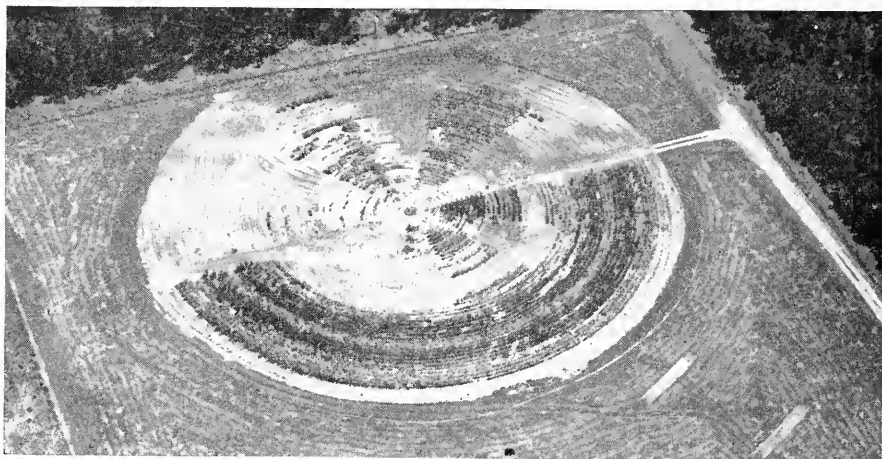
Although Stadler observed no mutations from *Wx* to *wx*, other workers have revealed that spontaneous mutations at this locus do occur. The rate is similar to that of *Sh*—> *sh*, approximately one or two per million gametes tested.

It should be noted that Stadler studied the mutations in the female gametes, and that all mutants observed were tested to make certain that they were gene

changes and not the loss of a short piece of chromosome. All mutants showing no sterility, which is characteristic of losses of a piece of chromosome, were regarded as gene changes. The very high rates of mutation from $R \rightarrow r$ and $CI \rightarrow ci$, 492×10^{-6} and 106×10^{-6} , respectively, are probably explained by the fact that the two "genes" involved are really complex loci, and that the mutation may have been occasioned by crossing-over within a compound locus, rather than by an intragenic change.

MUTATION RATE IN MICROSPOROGENESIS IN MAIZE

A technique similar to that employed by Stadler has been used by the author for the determination of both spontaneous and induced "mutations" in the developing pollen in maize. Plants dominant for several genes were irradiated by growing in a field with a radiation source of Co^{60} (Figs. 17-8 and 17-9). Pollen was collected from plants in the radiation field and



Courtesy of Brookhaven National Laboratory

FIG. 17-8 Airplane view of gamma field at Brookhaven National Laboratory. The radioactive Co^{60} source is placed in the center, with plants grown in concentric circles around it.

placed on silks of a recessive stock grown in a "clean" field, or one with no radiation. Four genes were studied intensively: $Su \rightarrow su$ in chromosome 4, $Pr \rightarrow pr$ in chromosome 5, $Y \rightarrow y$ in chromosome 6, and $Sh \rightarrow sh$ in chromosome 9. The su gene produces a sugary kernel, the kind we eat as sweet corn; pr a red rather than a purple (Pr) aleurone; y a white kernel, and sh a kernel that is deeply indented or shrunken (Fig. 17-10).

It was observed that radiation increased the mutation rates markedly over the spontaneous rate. Since the changes were induced in the pollen that has two sperm nuclei (one fertilizes an egg cell to form the embryo and the sec-

ond unites with two polar nuclei to form the endosperm), it was not possible to grow the resulting mutants to test them, as Stadler did. Indirect evidence indicated that most of the "mutants" were really losses of a short segment of chromosome, rather than an intragenic change. This was undoubtedly true for spontaneous mutants also observed in untreated plants where the mutation rate in microsporocytes was tested. Direct evidence regarding the types of changes produced was obtained by using a genetic stock marked for four loci



FIG. 17-9 Radiation field at the Blandy Experimental Farm, University of Virginia. The plants are "portable" (grown in pails) and are moved to the field for intense radiation from Co^{60} during short periods—usually one day. The radioactive source is well shielded by earth and concrete. The sky shine shield intercepts vertical radiation. The control house is in upper left center, 100 feet from the radiation machine.

in a single chromosome, No. 9. In this test, it was found that at least 95% of the changes were losses of a piece of chromosome, more than 75% being the loss of the whole arm.

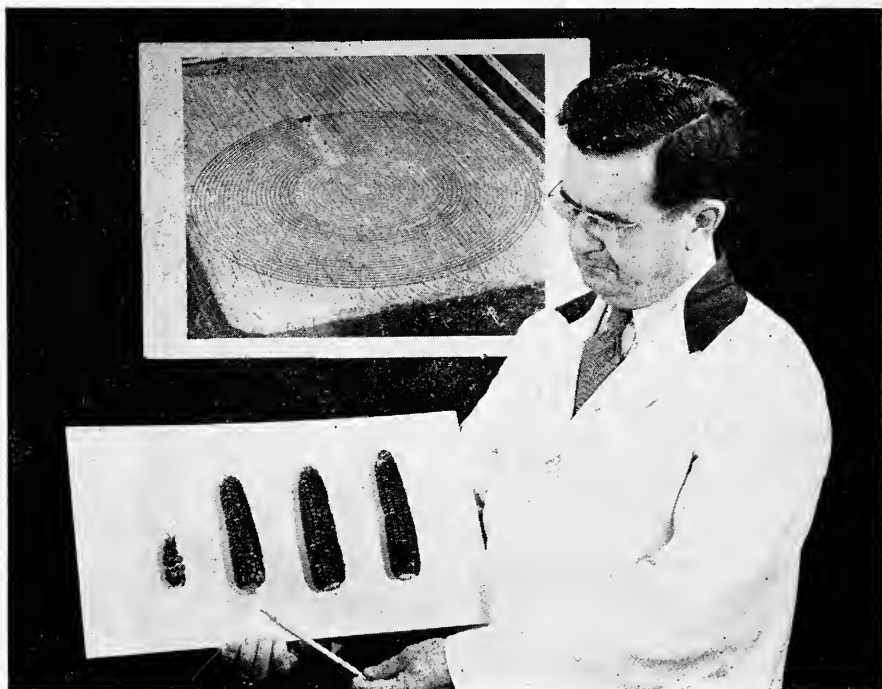
This experiment with maize is rather typical of other radiation studies. One of the chief effects is to break chromosomes. Phenotypic changes may result from such breakage, provided the stock is adequately marked genetically. The maize plant is an ideal tool for a cytogenetic study of radiation effects, since it is so well marked genetically, and the ten pairs of chromosomes are morphologically distinct.

Mutants produced by treating pollen (or the developing pollen) are affected in germinal tissue. Most, if not all, of the maize mutations induced in the pollen grains are of a more deleterious nature than spontaneous ones. This led Stadler to doubt that true intragenic mutations could be induced in maize pollen by ionizing radiation. Until lately, there was no evidence that mutations induced in the pollen by ionizing radiation were qualitatively the same as



Courtesy of Brookhaven National Laboratory

FIG. 17-10 Endosperm characters of maize in mutation experiments. Left to right: *A C R Pr Y Su Sh*, *A C r pr y su sh* parent stocks. The mutants next in order are *su*, *pr*, *sh*, *r*, *pr sh*, *pr su*, *r sh*, and *r su*.



Courtesy of Brookhaven National Laboratory

FIG. 17-11 Author with four ears of corn grown under continuous radiation. The background shows the original Brookhaven National Laboratory Co^{60} radiation field, the first ever established (1949). They (left to right) received 230, 113, 54, and 27 r per day

spontaneous mutants. However, in recent experiments, the author and his colleagues have succeeded in producing mutations from *Wx* to *wx* in maize. These fulfill all the requirements for intragenic changes. One critical test is whether the induced mutants will back-mutate to the original condition. The two waxy mutants (induced in premeiotic cells) mutated back to *Wx* (normal starch) with about the same frequency as the spontaneous *wx*.

MISCELLANEOUS RADIATION EFFECTS

By growing plants under continuous radiation, it is possible to observe various physiological effects as well as different genetic changes (Fig. 17-11). The most drastic effect observed is the killing of the plant. Lethal



Courtesy of Brookhaven National Laboratory

FIG. 17-12 Deleterious effects of X rays on growing tomato plants.

doses have been determined for a wide variety of plants, as well as the amount of radiation necessary to reduce the total growth seriously. The effects of chronic radiation are similar to those of acute radiation using X rays, whose effects can be seen in Fig. 17-12. This is an illustration of some of the work of Sparrow and his colleagues. They have made an extensive study of the amount of radiation that different species of plants will tolerate with little damage, and also of the amount necessary to damage seriously or to kill the various species (Table 17-2).

From Table 17-2 one can see a big difference in the sensitivity of various species of plants to radiation. In general, Sparrow has found that the plants with large chromosomes are the most susceptible. It is noted that the plants appearing at the top of Table 17-2 are the ones that have proved so adaptable as cytological material, mostly because of the large chromosomes.

The gladiolus plants proved to be extremely resistant to radiation, with a dose of 6000 r/day required to cause severe injury. Maize plants were intermediate. It should be noted that there are genetic differences within a species

Table 17-2. Tolerance of 30 Species of Plants to Chronic Gamma Radiation (Sparrow and Christensen)

Plant	Minimum Exposure (Weeks)	Effect at Indicated Dose Rate ^a (r units per day)	
		Mild	Severe
<i>Lilium longiflorum</i>	15	20	30
<i>Tradescantia paludosa</i>	15	20	40
<i>Tradescantia ohimensis</i>	15	35	65
<i>Vicia faba</i>	15	60	90
<i>Impatiens</i> sp.	18	60	90
<i>Coleus blumei</i>	13	100	240
<i>Melilotus officinalis</i>	14	100	240
<i>Nicotiana rustica</i>	15	100	300
<i>Datura stramonium</i>	7	110	360
<i>Gossypium hirsutum</i>	15	110	250
<i>Zea mays</i>	13	110	250
Dahlia (hybrid)	10	110	275
<i>Althea rosea</i>	12	120	250
<i>Luzula purpurea</i>	10	125	300
Chrysanthemum (hybrid)	18	140	250
<i>Canna generalis</i>	18	180	350
<i>Lactuca sativa</i>	7	180	600
<i>Chenopodium album</i>	15	250	450
<i>Antirrhinum majus</i>	18	250	400
<i>Lycopersicon esculentum</i>	15	250	400
<i>Xanthium</i> sp.	15	250	500
<i>Solanum tuberosum</i>	10	300	600
<i>Petunia hybrida</i>	10	300	700
<i>Lupinus albus</i>	12	400	—
<i>Kalanchoe daigremontiana</i>	12	400	800
<i>Allium cepa</i>	18	400	800
<i>Linum usitatissimum</i>	10	600	1100
<i>Digitaria</i> (crabgrass)	12	1000	1800
<i>Brassica oleracea</i> (broccoli)	10	1400	2500
<i>Gladiolus</i> (hybrid)	8	4100	6000

^a Dose rate is in roentgens/24-hour day; however, the actual dosage/day averaged about 90% of the dose rate shown.

for susceptibility to radiation. One maize hybrid showed comparatively little injury by radiation of 127 r/day, while another (Wf9 × 38-11) had its yield seriously reduced by as little as 110 r/day. Compare ears in Fig. 17-11 with those in Fig. 17-13.



Courtesy of Brookhaven National Laboratory

FIG. 17-13 Ears of field corn hybrid Wf9 \times 38-11. They (top to bottom) received 100 r per day, none, and 110 r per day. This strain was more sensitive than the one shown in Fig. 17-11.

CORRELATION BETWEEN DOSE OF RADIATION AND INDUCED MUTATIONS

In the original investigations on the induction of mutations in *Drosophila*, Muller had determined that doubling the dose resulted in approximately twice the number of mutations. Extensive studies by C. P. Oliver in this country and Timofeeff-Ressovsky in Russia very early showed that, for a considerable range in dosage (from 1000 to 10,000 r), the percentage of mutations was roughly proportional to the radiation. In other words, as the dosage of X ray increases, the percentage of mutations increases.

One of the most comprehensive tests, including dosages of radiation as low as 25 r, was made by W. P. Spencer, College of Wooster, and Curt Stern, University of California. They used the Muller-5 method described previously for the determination of sex-linked lethals. Their data are presented in Table 17-3 and also in a graph (Fig. 17-14).

It is evident from Table 17-3 and the graph of these data (Fig. 17-14) that the amount of mutations produced is directly proportional to the dose of radiation given. This holds for the lowest dose studied, 25 roentgens. These

Table 17-3. X Chromosome Lethals in *Drosophila* Produced by Different Amounts of X-radiation (data of Spencer and Stern)

Dose in Roentgens	No. of Chromosomes Tested	No. of Chromosomes with Lethals	Rate per 10^4 Gametes
0 (Control)	73,901	72	10
25	51,907	88	17
50	31,560	77	24
150	23,195	74	32
500	6,634	87	131
1000	6,977	147	211
2000	2,755	130	472
3000	2,029	132	651
4000	1,843	182	988

findings have been verified in a wide variety of organisms, including a mammal, the mouse. This is true only for gene mutations or changes that could be induced by a single ionization. For changes induced by two independent breaks induced simultaneously, a different type of dose response is found. If two breaks are required to produce a result, it is obvious that very low doses of radiation would have a disproportionately low percentage of changes. In many cases the low dose would cause not more than one break in a given cell. Thus there would be a low dose, below which no changes would be induced. This is the *threshold* effect, which is found for chromosomal events, but not for gene changes.

Recently, H. Bentley Glass and Rebecca Ritterhoff (1961) at the Johns Hopkins University conducted an experiment regarding the mutagenic effect of an extremely low dose of X-rays on the increase in the mutation rate of dominant bristle mutations in *Drosophila*. They employed a dose of only five roentgens, which in human beings would be comparable to the background rate over a 30-year period covering the most active reproductive time in man.

Experiments of this kind are extremely laborious. It is necessary to examine a great many flies, since the mutation rate is very low. The investigators examined more than a million flies, about half of which were controls with no radiation, the other half being progeny of the flies receiving five r of X rays.

From established dosage curves at 1000 and 2000 r, and assuming a linear proportionality of mutations to dose, they estimated that the increase in mutation rate would be 0.005 per cent, in comparison with a spontaneous rate of 0.04 per cent. In the control group of more than one half million flies, they obtained 283 mutations; in the irradiated group of more than one half million, they obtained 323 mutants. The overall control rate was 0.048 per cent, a little more than predicted, while their irradiated material showed 0.055, or an increase over the control of 0.007 per cent, a little higher than the estimated value, although barely significant.

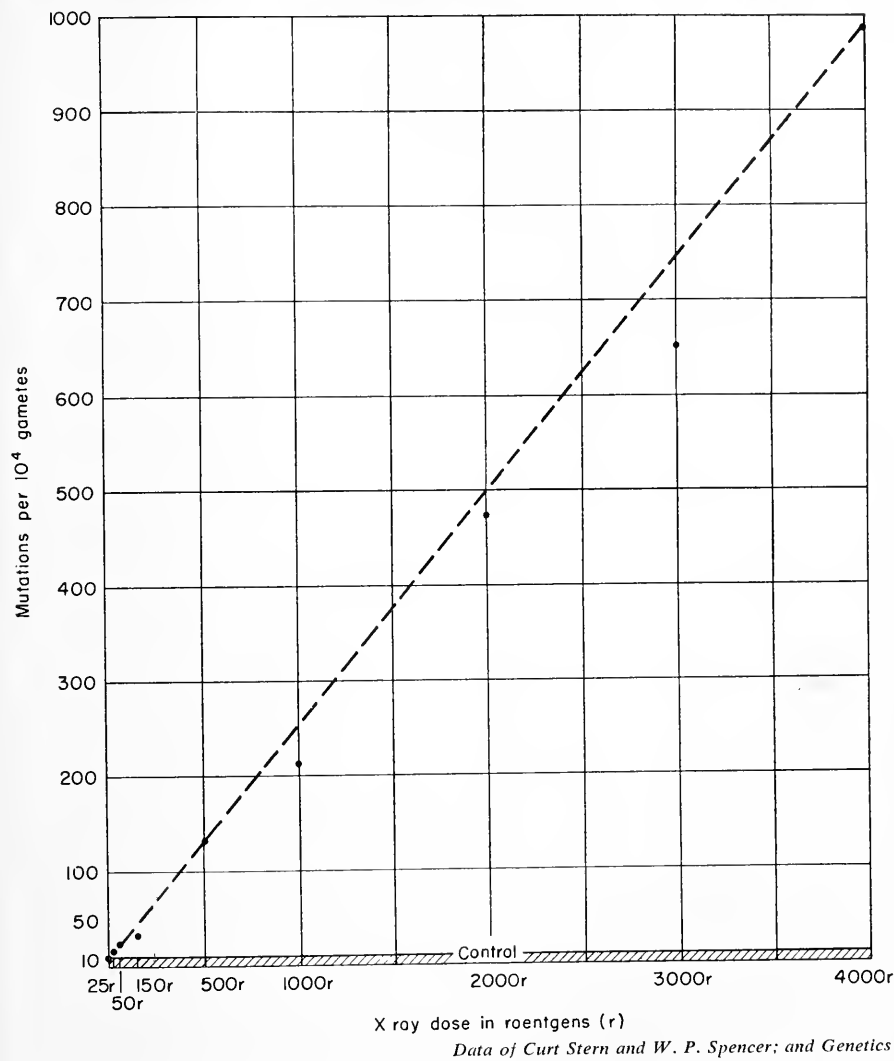


FIG. 17-14 Linear relationship of X ray dose in roentgens and rate of induced lethal mutations in X chromosome of *Drosophila*.

From their experiment they concluded that an acute dose of 5 r produces mutations at a rate linearly proportional to the effects at 1000 r and 2000 r, in the mature gametes of both sexes of *Drosophila*.

This extremely low dose (5 r) produces *proportionately* as many mutations as the much higher doses of 1000 r and 2000 r. Thus it is evident that there is *no safe dose* from a genetic standpoint. Any increase over the background rate will produce genetic damage. This is one of the reasons that geneticists

are so concerned that the human species receive no more radiation than is absolutely necessary during the reproductive years.

Since a roentgen delivered in a very low dose produces as many mutations as a roentgen delivered in a high dose, the total genetic damage to a population from exposing 1000 people to one roentgen would be approximately the same as if 100 people received ten roentgens, or ten people received 100. The total dose in roentgens determines the genetic damage. It is true the higher dose may produce more chromosomal events and also more sterility.

CHROMOSOMAL BREAKS INDUCED BY RADIATION

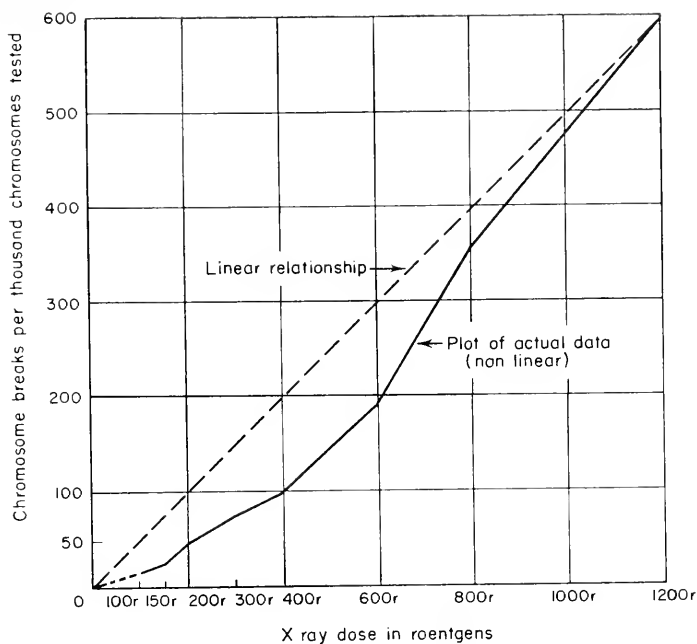
It was learned very early that ionizing radiation is effective in breaking chromosomes. One of the first experiments was that of Sax. He studied the chromosome breaks induced in *Tradescantia*, a plant much used in investigating the cytological effects of radiation. His data are found in Table 17-4 and are shown graphically in Fig. 17-15. It can be seen that the breaks

Table 17-4. Chromosome Breaks in *Tradescantia* as a Function of Dose of X Rays (data of Sax)

Dose in Roentgens	No. of Chromosomes Tested	No. Breaks	Rate $\times 10^{-3}$
100	2538	40	16
150	1896	48	25
200	1476	70	47
300	1626	120	74
400	3384	332	98
600	1446	275	190
800	2214	796	359
1200	1086	644	593

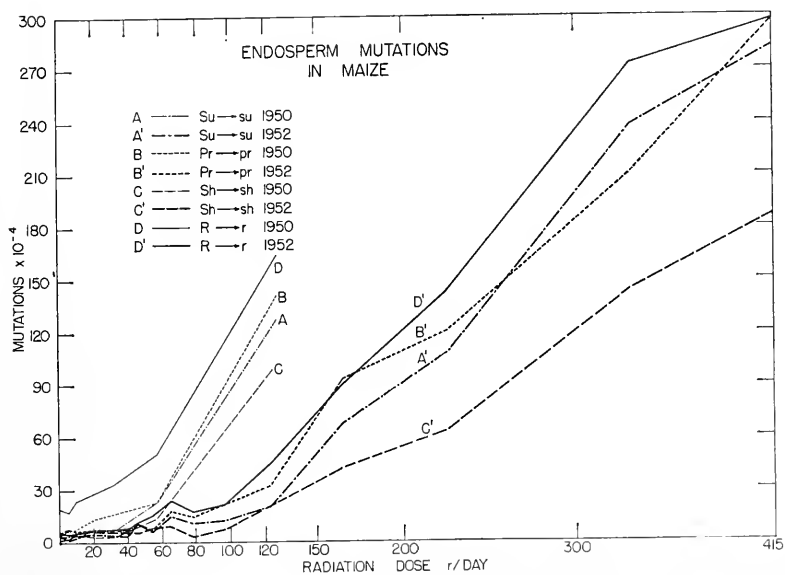
increase faster than the radiation. This is because the component of the breaks produced by two or more independent ionizations can be expected to increase sharply as the density of ionization increases, allowing more opportunity for two ionizations to occur in the same cell.

A similar curve was found when endosperm changes in maize were plotted against dose rate after plants had been exposed to chronic gamma radiation (Singleton 1954). (Fig. 17-16.) Below 40 r/d the induced rate of mutations was not appreciably greater than the control rate. At the higher doses of radiation, in excess of 100 r/d, the percentage of mutations rose more sharply than the increase in radiation dose. It was evident that at least part of the effects were chromosomal in nature. Later experiments have established the fact that perhaps 95% of the endosperm changes in maize are of this kind. Apparently this holds for spontaneous rates in the pollen also, giving a spuri-



Data of Karl Sax, and Genetics

FIG. 17-15 Exponential relationship of X ray dose and chromosome breaks in Tradescantia.



Courtesy of Brookhaven National Laboratory

FIG. 17-16 Dose response curve for induced endosperm mutations in maize. This resembles Fig. 17-15 more closely than Fig. 17-14, which indicates that a large component of endosperm mutations was caused by chromosome breaks.

ous spontaneous rate much higher in the male than in the female gametophyte. If only gene mutations are considered rather than "gene losses" through a missing piece of chromosome, the spontaneous rates in the male gametophyte are perhaps no higher than the female gametophyte.

OTHER MUTAGENIC AGENTS

Ultraviolet light is also an effective mutagenic agent. One of its limitations is its lack of penetration into any but very small objects. Stadler used ultraviolet light as a mutagen in maize pollen, the only part of the plant where ultraviolet can enter to produce mutations. He found that this agent induced mutations more closely resembling spontaneous ones than did the mutants of ionizing radiation. There was quite a high incidence of endosperm mutations, where half of the kernel was like the irradiated pollen parent and one half was of the mutant characteristic. This was evidence that one chromatid was affected without the other. Some of the ultraviolet induced mutants showed no sterility and seemed analogous to spontaneous mutants. Ultraviolet is known to break chromosomes, as do X rays. In maize at least, most ultraviolet induced deletions are terminal, in contrast with interstitial deletions which comprise most of the X-ray induced losses. Ultraviolet has been used extensively as a mutagen for bacteria and the lower forms, where penetration is no problem. The ordinary sterilizing lamp (wave length 2537 Å) is a good mutagenic tool. If too large doses are given the organisms are killed, which is the purpose of the manufacturer.

Certain chemicals are known to be mutagenic. The study of mutagenic chemicals was pioneered by Charlotte Auerbach, who worked with nitrogen mustard gas in Edinburgh, Scotland, during World War II. Her work at that time was classified, but later she was able to publish rather striking results, showing mustard gas to be highly mutagenic. Since that time many other chemicals, including such common ones as formaldehyde, have been shown to be mutagenic.

Demerec and his associates at the Carnegie Institution laboratory at Cold Spring Harbor, New York, have been active in the study of mutagenic chemicals on some of the lower forms.

Most of the mutagenic agents, whether radiation or chemicals, break chromosomes as well as produce genetic changes. In fact, one of the chief ways that ionizing radiation produces genetic changes is by breakage of the chromosomes.

MUTAGENICITY OF PEANUT, MUSTARD, AND CASTOR OIL ON CEREALS

An interesting experiment on the mutagenicity of some of the common vegetable oils, castor oil, mustard oil, and peanut oil was performed



*Courtesy of M. S. Swaminathan and A. T. Natarajan;
and Journal of Heredity*

FIG. 17-17 Earhead variations in the second generation of wheat plants grown from seed treated in oil. Above: (1) Reduced awns in the lower spikelets (castor oil), (2) long tipped (castor oil), (3) red glume (peanut oil), (4) red glume-speltoid (peanut oil), (5) speltoid (mustard oil), (6) lax (castor oil), (7) dense spike of erectoid mutant (peanut oil), (8) beardless mutant (peanut oil). Below: Control (extreme left) and erectoides mutants.

by Swaminathan and Natarajan in India (1959). They observed that it was common knowledge that some species with a high concentration of oil in the seed were more resistant to the effects of radiation than other species like the cereals, with low oil content. They reasoned that the cereals might be rendered more resistant if soaked in vegetable oils.

After the seeds were soaked in either castor, mustard, or peanut oil, they were germinated in sand. The results were startling. Instead of the oils imparting resistance to the cereals after radiation, the oils themselves caused a marked decrease in germination of the seeds. In addition, in root tips, also at meiosis, a high percentage of chromosomal irregularities were induced, as well as several mutations in the bread wheats (grown to maturity).

The reduction in germination was more pronounced in the species with the lower chromosome numbers, *Triticum monococcum* ($n = 7$) and *T. dicoccum* ($n = 14$). For example, germination was completely inhibited in *T. monococcum* by soaking six hours in peanut oil, while *T. dicoccum* (Emmer) showed only 38% germination. However the bread wheat *T. aestivum* ($n = 21$) had 46% germination after soaking not six, but 24, hours in peanut oil. The bread wheat was the only species in which mutations were induced.

The amazing discovery was that the mutation rate induced in *T. aestivum* by peanut oil was higher than that produced by X rays, fast neutrons P^{32} and S^{35} , nitrogen mustard, and the other vegetable oils used. The mutation frequency was 156% per plant progeny. Some of the mutant characters induced are shown in Fig. 17-17. Castor oil produced 61% and mustard oil 4% per plant progeny.

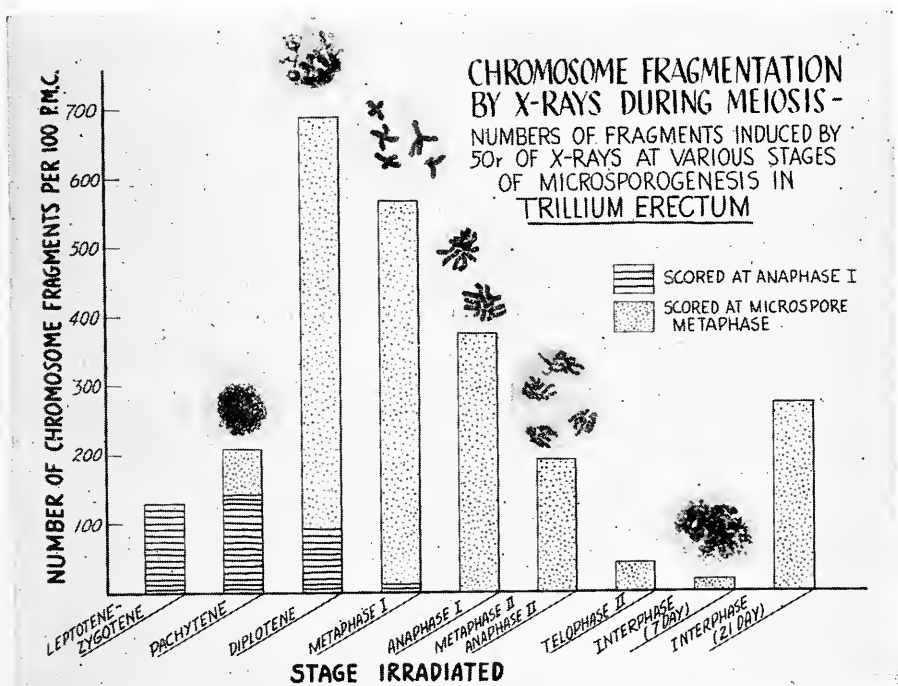
Recently two research workers at Washington State University, C. F. Konzak and R. A. Nilan, discovered that one chemical, diethyl sulfate, produces gene changes in barley without the concomitant chromosomal breakage usually accompanying such changes. This chemical and others under investigation may prove to be something that geneticists have been hunting for a long time—a mutagenic agent that can produce gene changes without breaking chromosomes.

DIFFERENTIAL SENSITIVITY OF CELLS TO RADIATION

Cells undergoing meiosis are extremely sensitive to radiation. This has been demonstrated in a wide variety of organisms, such as Trillium, mice, maize, and *Drosophila*. Sparrow and his colleagues investigated the sensitivity of Trillium cells undergoing meiosis to a given dose of radiation. His results are shown graphically in Fig. 17-18.

A similar relationship exists for *Drosophila* spermatozoa, as has been demonstrated by Mary Louise Alexander at the University of Texas. William Russell at the Oak Ridge National Laboratory has shown a similar condition for developing spermatozoa of mice. The same holds true for corn, illustrated

in Fig. 17-19. These data on corn were obtained by irradiating growing plants at different stages of development, from very young plants (before meiosis) to mature pollen. It was found that metaphase I was extremely sensitive to radiation, and that very few pollen grains survived a treatment of 1320 r at this stage. This is shown by the seed set, which is practically zero for pollen irradiated at metaphase I.



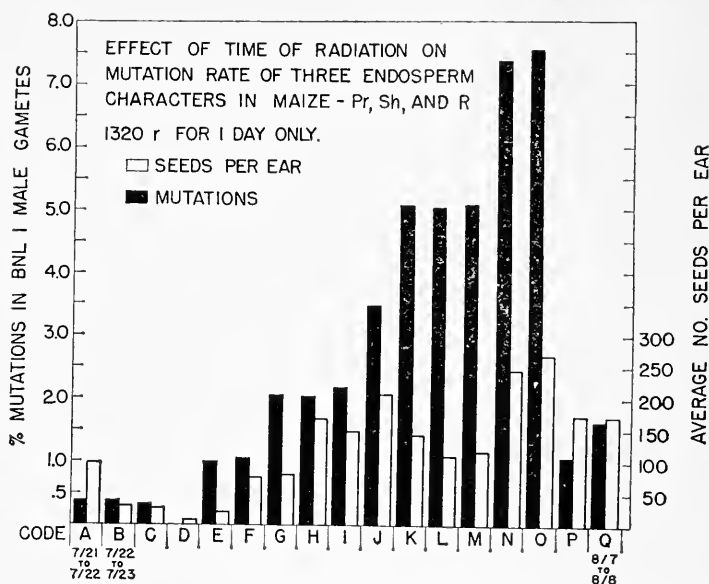
Courtesy of A. H. Sparrow; and Brookhaven National Laboratory

FIG. 17-18 Extreme differences in sensitivity of different stages of meiosis in *Trillium* to X rays.

There were a few mutations before meiosis. Most of the observed changes occurred in pollen samples irradiated afterward, but before pollen shedding. Practically all of the endosperm changes observed for these latter stages were due to losses of a portion of chromosome, rather than an intragenic change. Such changes have been induced when premeiotic cells were irradiated.

RADIATION-INDUCED GENETIC DAMAGE IN MICE

* Extensive mouse irradiation studies have been made by Russell and his wife, Liane Brauch Russell, at Oak Ridge. To obtain data comparable to that from *Drosophila*, maize, and microorganisms, it was necessary to grow



Courtesy of Brookhaven National Laboratory

FIG. 17-19 Differences in sensitivity of corn plants to gamma radiation. At meiosis (D) plants were extremely sensitive to radiation and produced few seeds.

Most mutant endosperms recovered three days before pollen shedding (O).

large colonies of mice numbering in the hundreds of thousands. This was a great undertaking. It required extensive laboratory facilities and large-scale housing for the animals. Fortunately, these were provided by the Oak Ridge

Table 17-5. Mutations of Specific Loci in Spermatogonia of Mice Induced by X Rays (data of Russell and Russell)

Dose in Roentgens	Region Irradiated	No. of Offspring	No. Mutations at 7 Loci	Mean No. Mutations per Locus per Gamete $\times 10^5$
0 (Control)	—	106,408	6	0.8
600	whole-body	119,326	111	13.3
0 (Control)	—	33,972	2	0.8
1000	posterior $\frac{1}{3}$ of body	31,815	23	10.3
0 (Control)	—	42,833	1	0.3
300	whole-body	19,449	13	9.6
300	posterior $\frac{1}{3}$ of body	20,959	12	8.2
Total controls		183,213	9	.7
Total X-rayed		191,549	159	11.9

National Laboratory. It is of the greatest importance to have data showing the amount of genetic damage in the mammal most closely related to man, of all genetical material studied extensively.

Dr. and Mrs. Russell investigated the mutation rate for seven specific loci. Males dominant for all the seven genetic markers were irradiated. Following a period of temporary sterility, they were mated to females recessive for the seven marker genes. Most of the X_1 progeny consisted of animals with the dominant phenotypes, as was expected. In rare instances animals with one of the seven different recessive phenotypes were observed. The frequencies of the recessive phenotypes in the irradiated progenies were compared with those of the controls with no radiation. The Russells' data for three separate experiments in mouse radiation (Russell and Russell 1959) are summarized in Table 17-5. They show a marked increase in the mutation rate as a result of radiation. However, the increases do not give a linear dose response relationship. The mutations at 300 r are greater than would be expected on the basis of the 600 r and the 100 r results.

In addition to acute exposure from X rays, animals were exposed to chronic gamma radiation from a Cs^{137} source (Table 17-6).

Table 17-6. Mutations at Seven Loci Induced in Spermatogonia of Mice by Chronic Gamma Radiation

<i>Dose in Roentgens</i>	<i>Intensity r/week</i>	<i>No. of Offspring</i>	<i>No. of Mutations</i>	<i>Mutations per Locus per Gamete $\times 10^5$</i>
0	—	66,107	6	1.3
86	10	18,973	4	3.0
516	90	10,446	1	1.4
861	90	12,937	6	6.6
Total irradiated		42,356	11	3.7

The data seem to indicate that the effects of acute irradiation may be more severe than chronic irradiation. The frequency of mutations induced by either X rays or gamma rays is higher in mice than in *Drosophila*. In estimating radiation hazards to human germ plasm, it seems wise to base estimates of damage on the highest mutation rate found in acute radiation of mice. It is the only mammal on which we have extensive data.

The Russells also found that the average litter size resulting from X-irradiated males was significantly smaller (5.55) than the controls (5.77). This would indicate a dominant deleterious effect in the male gametes irradiated in the spermatogonial stage.

POSSIBLE MEANING TO MAN OF MOUSE IRRADIATION DAMAGE

Some of the conclusions reached by the Russells regarding the effects of radiation of mice and the extrapolation of these results to man are as follows:

In view of the nature of the departures from linearity in the relation between mutation rate and dose, it would seem wise, in estimates of human hazards based on mouse mutation rates, to take the highest mutation rate obtained, namely, that observed in the experiment using a dose of 300 r of X rays. The induced rate in this experiment is 28×10^{-8} per roentgen per locus.

Deleterious effects on the viability of first generation offspring of irradiated mice have been found in several experiments. Dominant effects may comprise an appreciable part of the total genetic damage from radiation-induced mutations.

The low frequency of translocations in the offspring of females indicates that this particular type of genetic effect may be unimportant as a hazard.

The low specific locus mutation rate obtained in offspring of female mice exposed to 261 r of chronic gamma-irradiation raises considerable hope that this finding may apply to the human ovary.

USE OF INDUCED MUTANTS IN PLANT IMPROVEMENT

Muller's classic paper "Artificial Transmutation of the Gene," which was published in 1927, pointed out the possibilities for the use of this new technique of X radiation in developing superior plants. In his conclusion Muller stated, "Similarly for the practical breeder it is hoped that the method will ultimately prove useful."

It was not long after this statement that plant breeders initiated research to develop new and improved varieties by means of X radiation. Two of the pioneers were the late Herman Nilsson-Ehle, the director of the Swedish Seed Association at Svalof and head of the Genetics Institute of Lund University, and A. Gustafsson, one of his students.

They worked with cereals, primarily with barley, and were able to produce varieties with stiffer straw, a more dense head, and higher yield than the parent seed. Their method was to irradiate seed, save many heads on the R_1 plant, and in succeeding years grow a single progeny from each head produced on the R_1 plants. Many mutants for chlorophyll characters, such as albino, virescents, abescents, etc., were found in those single head progenies. Also, a few mutants with short straw and dense heads occurred. These were called *erectoides*. See Fig. 17-17 for comparison of normal and erectoides. Some of them proved to be higher yielding than the parental strain. Thus the plant breeders were able to demonstrate that the new method proposed by Muller was actually feasible.

Only a very small percentage of all the mutants was useful in plant breed-

ing. Gustafsson has estimated that less than one in a thousand mutants produced ($<.1\%$) may be useful in plant breeding. Probably a sizeable number result from loss of a bit of chromosome, since it is known that ionizing radiation breaks chromosomes rather readily. Such losses usually produce some sterility in plants and these would be expected to be less vigorous than the normal parent. So it is not surprising to find the number of beneficial mutants less than one in a thousand.

From a plant breeding standpoint there is a tremendous difference between 100% deleterious mutants and 99.9%. The one tenth of one per cent offers great promise for the plant breeder. By producing mutants in large numbers, it is possible to select the few that may have potential as new and improved varieties. Since the plant breeder can work with large populations and discard freely, he has a chance of saving the rare beneficial mutant. Even with conventional plant breeding, it is almost axiomatic that success is proportional to the amount of material discarded. This is even more likely to be true of one using mutagens.

In the brief section of this chapter on alteration of the germ plasm, it is not possible to list all of the plant breeding achievements with mutagens. The most important may be found in the supplemental reading at the end of the chapter. Atomic radiation has been particularly helpful in producing new and improved types of cereals.

GREGORY'S MILLION IRRADIATED PEANUTS

One of the most successful plant breeding projects using radiation is that of Walton C. Gregory (Fig. 17-20), working with peanuts at the North Carolina Agricultural Experiment Station, Raleigh. His labors demonstrate the point that large numbers must be used. He and his co-workers examined more than a million plants grown on 64 acres in the segregating generation, the R_2 following X radiation. A wide variety of mutants was produced, including a few that were higher yielding than the parents. Years of experimentation, which included backcrossing to the original parent, were required before the present superior types of peanuts were developed.

It should be emphasized that plant breeding using radiation or other mutagens is not a short cut to the production of new and superior types. Usually it takes about as long to produce a variety by this method as by the older conventional one of crossing, examining the F_2 generation, backcrossing, further testing, and, finally, increasing the seed.

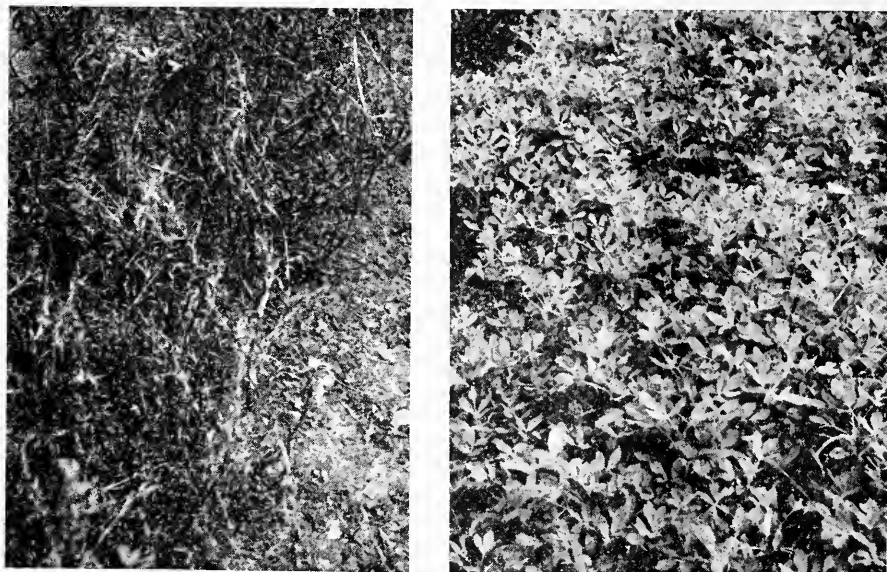
One advantage of using a mutagen is that a stable crop like peanuts can be



FIG. 17-20 Walton C. Gregory, successful radiation peanut breeder.

made to mutate, so that the plant breeder has a greater wealth of genetic variability with which to work. This is more important for self-pollinated plants, such as peanuts, which have eliminated most of their genetic variability through generations of self-fertilization. (See Fig. 12-10 for the effects of self-fertilization.)

The plant breeder himself must provide some variability if he is to have an opportunity for selection. He can do this by crossing with types having the desired traits (if these are available) or by the use of some mutagen to alter the germ plasm and produce changes. Gregory provided the variability in peanuts by having a considerable quantity of seed X-rayed at the Oak Ridge National Laboratory (Fig. 17-21).



Courtesy of W. C. Gregory

FIG. 17-21 Induced mutant in peanuts, showing resistance to leaf spot disease (right), in contrast to parental type almost completely defoliated.

One of the desirable mutants Gregory isolated from the populations from X-rayed seed was a type resistant to a serious leaf spot disease (Fig. 17-22). Radiation is particularly effective in producing disease resistance in crops. This is not because the resistant types are any more prevalent than other mutants, but simply because it is so easy to select them. If a susceptible type is irradiated and the R_2 generation of a million or more plants can be grown *in an environment where the disease is present*, the disease eliminates all the susceptible plants, leaving *only* the resistant types. Even if the rate of production of resistant mutants is as low as one in 100,000 or one in 1,000,000, the resistant type can be isolated simply by growing large populations. Mutagenic breeding works well for plants, but would be far too costly for animals.



Courtesy of W. C. Gregory

FIG. 17-22 Several mutant types of peanuts induced by X rays.

MICROSURGERY BY RADIATION

Radiation may be used to "cut out" a piece of chromosome from one species and transfer it to another. This approach has been used successfully by two researchers, E. R. Sears of the United States Department of Agriculture and the University of Missouri, Columbia, and Fred C. Elliott, then at Washington State College, Pullman. Sears, who pioneered the method, was able to transfer leaf rust resistance from a wild grass, *Aegilops umbellulata*, to wheat. First he made an F_1 hybrid, irradiated it to produce translocations, and then selected wheatlike plants that had the resistance of the wild grass. Without radiation the translocation rate would have been so low that this operation would have been made virtually impossible.

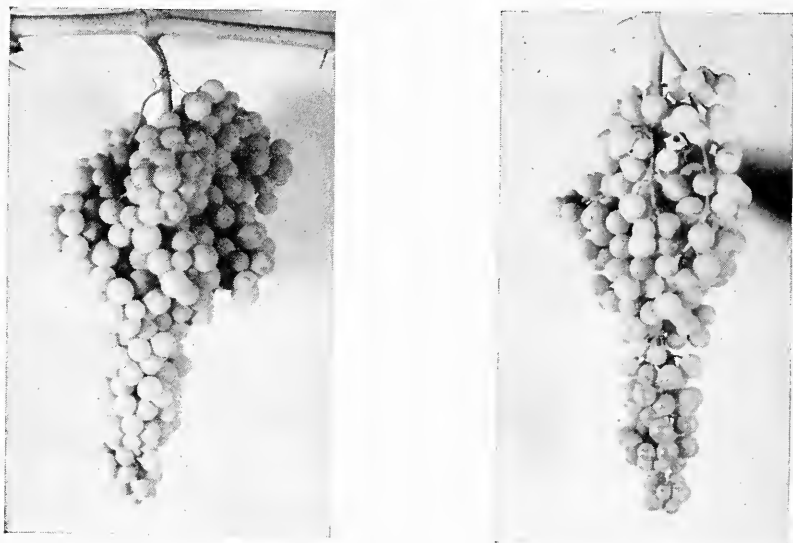
Elliott was able, through radiation, to transfer stem rust resistance from tall wheat grass, *Agropyron elongatum* ($2n = 70$), to a hexaploid wheat ($2n = 56$). In addition the new resistant wheat had red glumes, not found in either parent. This condition may have arisen as a result of deletions affecting modifier or suppressor relationships, or as a position effect.

The success of this microsurgery technique depends upon the ability of radiation to break chromosomes, one of the chief characteristics of ionizing radiation. The radiation is able to break the chromosome in more than one

place. By breaking it twice very close together, it simply "cuts out" a minute piece of chromosome, which is then transferred to a chromosome of the desired species. Since this piece of chromosome carries resistance to disease, this quality of one wild species is transferred to the cultivated species. This method undoubtedly will be used more extensively by plant breeders in the future.

INDUCED STERILITY BENEFICIAL

Although any induced sterility is not usually considered beneficial, it has been useful in some ways to the plant breeder. Richard Caldecott and others (1959) have demonstrated that following radiation, oat varieties



Courtesy of H. P. Olmo

FIG. 17-23 Induced mutant of grapes with loose clusters (right) in comparison with compact parental type (left). The sterility induced is beneficial in this case.

had considerable pollen sterility. They were rendered more susceptible to cross-pollination, and some of the resultant cross-pollinated types were resistant to stem rust. J. M. Pochlman (1960) at the University of Missouri has suggested the use of radiation-induced sterility as a means of producing mass hybridization where artificial crossing is slow and tedious. This method facilitates recurrent selection in normally self-pollinated crops.

Another case of beneficial sterility was reported by H. P. Olmo of the University of California at the 1960 Symposium on Mutation and Plant Breeding at Cornell University. Many commercial *Vitis vinifera* varieties of grapes set so many flowers that fruit clusters are too compact at maturity, resulting in ruptures that lead to fruit spoilage. A new seedless variety, Perlette (Fig. 17-23a), sets so heavily that it requires expensive thinning by hand. Follow-

ing irradiation, one mutant produced uniform, but loose, bunches because of induced sterility. This selection has shown sufficient promise to be introduced for trial as a possible new commercial variety (Fig. 17-23b).

X RAY-INDUCED MUTANT IN HIBISCUS PERMITS MIGRATION NORTHWARD

M. Pfluge Gregory and her husband, Walton C. Gregory, reported at the Cornell symposium (1960) an interesting radiation study. An X ray-induced mutation in *Hibiscus sabdareffa* enabled the plant to bloom and set fruit 1000 miles north of its normal habitat. This plant has a bright red calyx used in sauces, jellies, and drinks. Normally, it grows in southern Florida and is ready to harvest in December. In North Carolina, when sown in May, it does not initiate flower buds until October. It is a plant liking a short day, and initiates flower buds only when the days grow short. By X-raying the seeds (15,000 r) and growing a large population of segregating progenies in the X_2 generation, the investigators produced two lines of plants that flowered in August at Raleigh, North Carolina. Thus by the use of X rays the Gregorys have developed a Hibiscus capable of growing 1000 miles north of its normal home, and one which is 45 to 60 days earlier in flowering.

INDUCED SOMATIC MUTATIONS

Also in plants propagated asexually, mutagenic breeding has been used successfully to produce new types. By cuttings and grafts, the breeder's results can be established immediately as new varieties. Many of our fruit varieties have arisen by a spontaneous somatic mutation, commonly known as a *bud-sport* by the horticulturist. Witness the Red Delicious and the Double Red Delicious apples, the pink dogwood, and other types that have arisen from a single bud mutation. These developed into a whole branch, and grafting wood was obtained for the production of new varieties.

BROOKHAVEN GAMMA GARDEN, COOPERATION IN PEACEFUL USES OF ATOMIC ENERGY

Many woody plants have been grown in the gamma garden of the Brookhaven National Laboratory (Fig. 17-8), a cooperative venture with the several state agricultural experiment stations near the Brookhaven Laboratory. The various states supply the material which is grown for a time in the radiation field and then taken back home for study. The New Jersey experiment station at Rutgers, long interested in breeding peach varieties, planted several in the Brookhaven gamma field. After irradiation for one or more growing seasons, some of these peach trees were replanted at Rutgers. A few new types which developed were: (1) a variety a week earlier than the

parent, (2) one a week later than the parent, and (3) a type having more red coloring in the flesh. All three are potential new varieties and they can be propagated by grafting.

In other radiation experiments, apple varieties have been developed with greater or less red color in the skin. Also mutants with a russet or roughened skin have been induced from a variety normally having a smooth skin. Differences in flower type and color have been found in carnations. It seems likely that almost any mutant that has arisen spontaneously can also be produced by means of radiation or other mutagens. Furthermore, the frequency of new types under the influence of a mutagen is considerably higher than the spontaneous mutation rate.

SOMATIC MUTATIONS IN CARNATIONS

Gustav Mehlquist at the University of Connecticut, Storrs, has made extensive use of radiation in developing new varieties of carnations. He works in cooperation with the Brookhaven National Laboratory and has irradiated a large amount of material in the Co⁶⁰ gamma radiation field. Shapiro (1961*) reports Mehlquist has produced 21 mutants of the William Sim variety by radiation. Six of these (three white and three light salmon) are in limited commercial production. One mutant, R. No. 1, is a pure white clone with none of the red sinus blotches found on the blossoms of the White Sim. R. No. 1 was distributed in 1961. One additional white carnation and two light salmon look sufficiently promising for commercial production.

SOMATIC MUTATIONS BY CHANGE IN CHIMERAL CONDITION

The foregoing examples of somatic mutations probably represent gene changes, or at most the loss of a very small piece of a chromosome. It is also possible to produce changes that resemble somatic mutations by the alteration of an existing *chimera*, which is a mixture of two different kinds of tissue. In the White Sim carnation, the white color is due to a single layer of cells, the epidermis, while the tissue beneath is genetically red. This variety breeds as red from seed-grown progeny.

In radiation experiments, White Sim was grown in the cobalt-60 radiation field at Brookhaven National Laboratory (Richter and Singleton, 1955). At the end of the summer the plants were removed to the greenhouse for the winter. In several cases whole branches produced red flowers on white-flowered plants (Fig. 17-24). Further analysis of this phenomenon was made by Sagawa and Mehlquist (1957). They revealed that the change was brought about by a destruction of the epidermal layer, which allowed the tissue below to replace the epidermis and to express the red color, rather than the white. This is pictured dramatically in Fig. 17-25.

* Testimony before Congressional Committee, March 28, 1961.

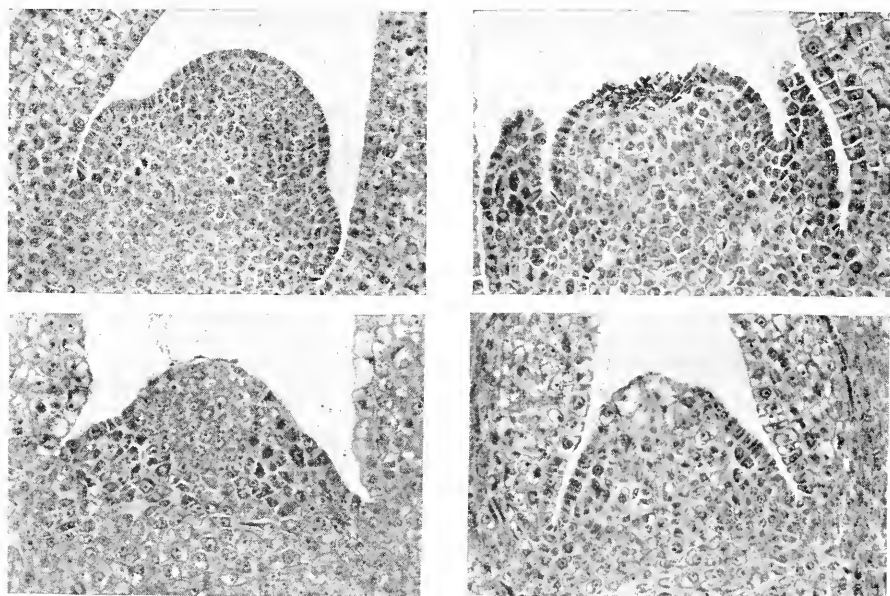
The mystery of the frequent occurrence of the "red mutant" in the White Sim carnation was solved by Sagawa's careful analysis of the morphology of X-rayed buds of the plant. Although this change in the flower color resulted from the uncovering of an existing periclinal chimera, the mutant in asexual propagation continued to be red and could be classified as a new variety. Thus



Courtesy of Brookhaven National Laboratory

FIG. 17-24 Red flowering branch on white carnation plant (center), the result of growing in gamma field. This type came from a change in the chimeral condition.

radiation produced a new type by altering the morphology of the flower, as well as by a true gene or chromosomal change. Regardless of the nature of the change, the "red White Sim" could be classified as a new variety similar to the William Sim, a red variety, from which the White Sim arose originally by spontaneous mutation. The experiment represents but another way in which radiation may be useful to the plant breeder, one of the many examples of the peaceful uses of atomic energy.



Courtesy of Y. Sagawa; and Brookhaven National Laboratory

FIG. 17-25 Normal bud of White Sim carnation (top left) with three buds that were X-rayed. The X rays destroyed epidermal cells (top right), allowing tissue beneath, which is genetically red, to determine color of the flower. Several branches so treated bore all red flowers instead of the normal white.

CONCLUDING REMARKS

Man has been interested in changing the germ plasm of organisms experimentally for almost as long as he has been aware that the germ plasm is separate from the somatic cells constituting most of the individual.

In 1892 Weismann proposed the theory that the germ plasm was really separate from the soma plasm and not subject to change by environmental conditions. Three years later, William Roentgen discovered the X ray, an event that was to have profound effects throughout the scientific world. Altering the germ plasm was one. This tool was used by Mavor in the early 1920's to induce hereditary changes in *Drosophila* caused by nondisjunction. It was the means used by Muller and Stadler a few years later to demonstrate unequivocal hereditary changes in *Drosophila*, barley, and maize.

With the advent of atomic energy and the marked increase in the use of radiation from isotopes after World War II, the number of induced hereditary changes in all sorts of organisms "mushroomed" almost like the cloud from the atomic bomb itself. Much has been learned regarding the induced changes and more knowledge has accumulated regarding the nature of the spontaneous mutation. The induced changes are a mixture of chromosomal and intragenic

mutants. Perhaps there is a preponderance of the former with ionizing radiation.

Mutagenic chemicals are being investigated intensively following the pioneer work of Auerbach during World War II. Evidence is accumulating that some of the mutagenic chemicals are able to produce intragenic changes without the chromosomal damage inflicted by ionizing radiation.

Geneticists soon realized that ionizing radiation may be a serious genetic hazard for the persons operating X ray equipment or patients receiving treatment. Even low doses produce their share of genetic damage. A roentgen of radiation delivered in a low dose produces as much genetic damage as a roentgen delivered in a high dose. The greater the total amount of radiation, the greater the genetic damage. From a population standpoint, one roentgen delivered to 1000 individuals produces as many genetic injuries as 10 roentgens to 100 individuals, or 100 roentgens to 10 persons.

The concern of the geneticist for undue amounts of radiation received by the human population has been translated into action. The maximum permissible dose permitted by workers in atomic energy has been gradually lowered. X ray machines have been improved with better screening to prevent undue exposure. Shoe-fitting machines using radiation have been outlawed in most states.

Atomic energy is here to stay, and the use of radiation will increase. Consequently, it is prudent to know the dangers and to take necessary precautions against undue exposure. It would not be wise to ban the use of all X rays and radioactive materials. The risks must be weighed against the potential benefits. The geneticists have an important role to play in advising means and methods of using radioactive sources, so that the precious germ plasm of the human race will not be seriously endangered.

The new tool of atomic energy should be handled with caution, but it is going to be used anyhow. What we need is perspective and common sense.

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PROBLEMS

17-1. Define or describe the following:

acquired character
 albescent
 albino seedling
 atomic energy
 balanced lethal
 beneficial mutant
 chemical mutagen
 chimera
 CIB method

Cy-L-Pm *Drosophila*
 detassel
 differential sensitivity
 endosperm mutation (maize)
 experimental alteration of germ
 plasm
 induced chromosome break
 inversion
 ionizing radiation

lethal mutation	spontaneous mutation
microsporogenesis	threshold
microsurgery by radiation	transmutation
Muller-5 method	virescent seedling
mutagenic	X ₁
somatic mutation	X ₂

17-2. Identify the following scientists, giving a major contribution with an approximate date.

Auerbach, Charlotte	Olmo, H. P.
Caldecott, R. S.	Roentgen, William
Castle, W. E.	Russell, W. L.
Demerec, M.	Sax, K.
Gager, C. S.	Sparrow, A. H.
Glass, H. B.	Spencer, W. P.
Gregory, W. C.	Stern, C.
Konzak, C. F.	Stadler, L. J.
Mavor, J. W.	Swaminathan, M. S.
Muller, H. J.	Weismann, A.

17-3. Show by a diagram how the *CIB* method operates to detect lethals induced in the X chromosome of *Drosophila*.

17-4. Likewise show how the Muller-5 method operates for detecting induced lethals in the X chromosome.

17-5. Show by a diagram how the Curly-Lobe-Plum (*Cy-L-Pm*) balanced lethal system operates to detect all mutants, visibles, lethals, semi-lethals, and sub-vitals in the second chromosome of *Drosophila*.

17-6. In a radiation experiment with *Drosophila* (see Table 17-3 and Fig. 17-4), which of the following would produce greater genetic damage to a population of infinite size?

- (a) Exposure of 2000 male flies to a dose of 25 r.
- (b) Exposure of 1000 males to a dose of 50 r.
- (c) Exposure of 100 males to 500 r.

17-7. In a cytological examination of induced breaks in *Tradescantia* (see Table 17-4 and Fig. 17-15), which of the following would produce the greatest total number of breaks?

- (a) Exposure of 1000 cells to a dose of 100 r.
- (b) Exposure of 250 cells to a dose of 400 r.
- (c) Exposure of 125 cells to a dose of 800 r.

17-8. In Table 17-1 the spontaneous mutation rate for *su* and for *y* was for each approximately two per million gametes. How many kernels would you have to examine to expect to find one spontaneous mutant for both *y* and *su* in the same kernel?

17-9. Radiation increases the frequency of mutations. For example, following chronic gamma radiation for 123 r per day the mutation rate for *sh* in chromosome 9 and *su* in chromosome 4 of maize were each 21 per 10,000 gametes tested. How many kernels would you have to examine to find a mutation for both *sh* and *su*?

17-10. Suppose you were also studying the mutations for *c* which too is in chromosome 9 approximately three crossover units from *sh*. With what frequency would you expect to find a *c sh* mutant, assuming the mutation rate for *c* alone to be equal to that for *sh* with the same amount of radiation?

Biochemical Genetics

IN THIS CHAPTER we are concerned with knowledge that has accumulated mostly during the last 20 years regarding the inheritance of metabolic deficiencies in lower organisms. Similar metabolic deficiencies in man will be discussed in Chapter 19. Chronologically, it might seem better to reverse the order, since we have known of the inheritance of metabolic diseases in man for a much longer time, almost as long as we have had a science of genetics. In 1902 the English physician Sir A. E. Garrod pointed out that alkaptonurea was a deficiency caused by a recessive gene. In 1909 he published his classic treatise "Inborn Errors in Metabolism."

I shall depart from the chronological order to present information gained from inducing "errors in metabolism" in lower forms, particularly in *Neurospora*, bacteria, and bacteriophage. It is through the study of these organisms that precise information regarding the inheritance of metabolic deficiencies has been obtained. Our knowledge of the nature of the gene, and of the way the gene produces its effects, also has been enhanced by such research.

We have become so familiar with the biochemical genetics of *Neurospora* and of bacteria, that these organisms are genetic tools as solidly entrenched as the long-studied *Drosophila* and maize. Actually the period of such biochemical genetics covers only a score of years. It was in December, 1941, that a piece of research startled the 10th annual meeting of the Genetics Society of America, held in Dallas, Texas. (This was but 14 years after Muller presented his classic paper in 1927 at a meeting of the American Association for the Advancement of Science in Nashville, Tennessee.) The paper reported biochemical mutants in *Neurospora* and presented the research of a now-famous team of investigators, Beadle (Fig. 18-1) and Tatum (Fig. 18-2), both of whom were then at Stanford University. Beadle was a geneticist, and Tatum a biochemist. Tatum is now a leading geneticist, as well as a biochemist. The

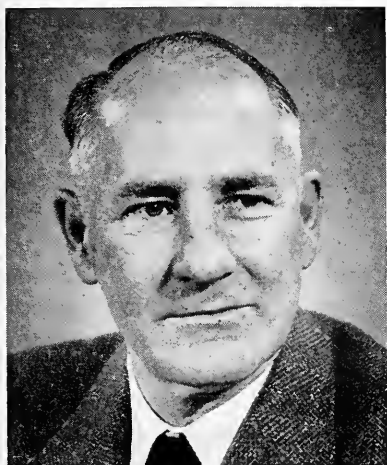


FIG. 18-1 George W. Beadle, pioneer in biochemical genetics of *Neurospora*.



FIG. 18-2 Edward L. Tatum, pioneer in biochemical genetics of *Neurospora*.

title of their paper was "Genetics of Biochemical Characters in *Neurospora*." The abstract is found in the *Records of the Genetics Society of America*, Vol. 10. Since it is only one paragraph long, we reproduce it below in full, with permission of the authors.

GENETICS OF BIOCHEMICAL CHARACTERS IN NEUROSPORA

From material X-rayed prior to meiosis, single ascospore cultures are grown on a medium to which is added, in the form of yeast extract, as many as is practicable of the substances normally synthesized by the organism. Such strains are subsequently tested for loss of synthetic abilities by transferring them to "minimal" media containing inorganic salt (ammonia nitrogen), a carbon source (sugars, starch or fat have been used in various tests), and biotin (the one required growth factor that cannot be synthesized by the normal strains). Induced loss of synthetic ability is indicated by failure to grow normally on such minimal media. Among the mutants obtained in this way are: (1) unable to synthesize vitamin B₆ (pyridoxine); (2) unable to make the thiazole half of vitamin B₁ (thiamine), and (3) unable to make para-aminobenzoic acid. Each of these differs from the normal by a single gene, and each is made indistinguishable from the normal by supplying it with the particular substance that it can not synthesize. These facts are consistent with the assumption that each of the genes involved is concerned with the control of one, and only one specific chemical reaction. Other mutants, not yet investigated as regards inheritance, are characterized by loss of ability to synthesize a growth factor different from any vitamin known to the authors, loss of ability to utilize fat as a carbon source, and a number of other ways. *The general approach offers promise both as a method of learning more about how genes control specific processes and as a means of studying biochemical processes as such.* [Italics are mine. W.R.S.]

This marks the beginning of the intense study of biochemical mutants in a wide variety of lower organisms. It is satisfying in studying a comparatively new science like genetics that we know so precisely the milestones, or turning points in its development. Surely 1941 was a memorable year in the science of genetics, just as were 1865 (Mendel's discoveries), 1900 (rediscovery of Mendel's paper by Correns, De Vries, and Tschermak), 1911 (Morgan's explanation of linkage), 1916 (discovery of nondisjunction by Bridges), and 1927 (Muller's induction of mutations with X rays).

Before discussing biochemical genetics in detail, it may be well to examine another piece of genetic research that led, in a way, to the unique approach of Beadle and Tatum. This was eye transplantation in *Drosophila*, conducted by Beadle in collaboration with Boris Ephrussi in Ephrussi's laboratory in Paris. Beadle has stated that it was the frustration with the eye color transplantation research in *Drosophila* that led to a series of studies by him and Tatum on the gene control of biochemical reactions in the red bread mold, *Neurospora*. Fortunately, the genetics of *Neurospora* had been carefully worked out by Bernard O. Dodge of the New York Botanical Garden. Dodge's work has already been discussed in Chapter 3.

EYE COLOR TRANSPLANTATION IN DROSOPHILA

Eye primordia taken from the larvae of one genetic type were removed and transplanted to the body of larvae of different genotypes. The interaction of host and transplant were then observed after the larvae had developed into adult flies (Table 18-1).

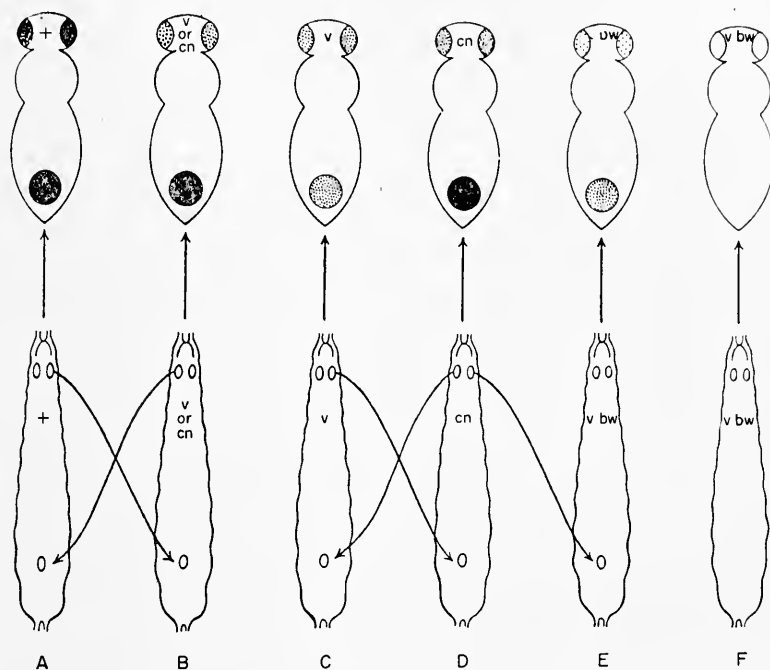
Table 18-1. Color of Eye Primordia Developing in Hosts of Different Genotypes

Eye color of host	Eye color of donor	Color transplanted eye	Diffusible substances
wild w^+	w	w	absent
white (w)	wild w^+	w^+	w^+ self-sufficient
wild w^+	v or cn	w^+	present in host
v or cn	wild w^+	w^+	w^+ self-sufficient
vermilion (v)	cinnabar (cn)	v	no cn^+ substance in host
cinnabar (cn)	vermilion (v)	w^+	v^+ substance in host

It can be seen from the table and from Fig. 18-3 that wild-type primordia (discs) are capable of producing full color in any cellular environment whether white (w), cinnabar (cn), or vermilion (v). In other words, they are self-sufficient, so that full normal color develops. Also, white eye discs produce white eyes in any cellular environment. No diffusible substance in a wild-type host is capable of producing color in a white eye disc transplant.

A contrast is noted when either cinnabar (cn) or vermilion (v) eye discs are transplanted into a wild-type host. In these cases, apparently, there is a

diffusible hormone-like substance, most likely an enzyme, in the wild fly. This is capable of restoring the genetic block of either the recessive *v* or *cn* eye discs, so that the transplanted recessive-type disc produces full color of the wild-type eye.



Courtesy of B. Ephrussi; and Quarterly Review of Biology

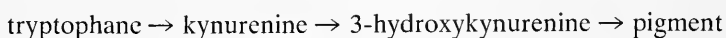
FIG. 18-3 Eye transplantations in *Drosophila* have demonstrated the order in which the genes *v*⁺ and *cn*⁺ act in sequence to produce wild-type eye. (Above) Adult flies containing differentiated implants; (below) host and donor larvae. See Table 18-1.

Reciprocal transplants between cinnabar and vermilion flies give additional information as to where the genetic block occurs. Is the genetic block that produces a vermilion fly at an earlier stage in the biochemical synthesis of full wild type of eye color, or does it occur after the genetic block that produces cinnabar?

When a cinnabar eye disc was transplanted into a vermilion fly, the cellular environment was able to transform the disc only to a vermilion and not to a wild-type eye. In the reciprocal transplant, however, when a vermilion eye disc was transplanted into a cinnabar host, the host apparently had some *v*⁺ diffusible substance that was able to transform the vermilion transplant into a fully colored eye.

These genes are concerned with subsequential steps in the biochemical synthesis of hormone-like materials that are utilized in production of eye

pigment. They are concerned with synthesis of products formed from tryptophane. Full pigment ommochrome is formed in the following sequence:

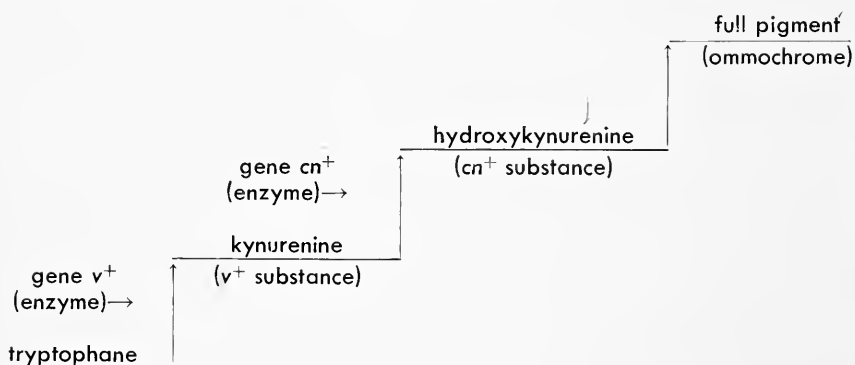


The recessive gene *v* blocks the formation of kynurenine, while *cn* blocks the transformation of kynurenine to 3-hydroxykynurenine. Since the formation of these chemical products is in sequential order, it is apparent why there is a difference between reciprocal transplants between *v* and *cn*.

A crude analogy with this situation might be visualized in the production of starch in a corn kernel. Once the starch is formed, it can be processed and converted into sugar. If no starch is formed, there is no possibility of converting it to sugar. The vermilion gene *v* in *Drosophila* inhibits the production of kynurenine. This substance is the raw material that the wild allele of the *cn* gene (*cn*⁺) converts into hydroxykynurenine in the sequence of full pigment production.

The metabolic steps involved in the production of full color are shown in Table 18-2.

Table 18-2. Sequential Steps in the Production of Full Eye Pigment of Wild-type *Drosophila*



BIOCHEMICAL MUTANTS IN NEUROSPORA

The main thesis of the work of Beadle and Tatum was that the many steps in the biochemical synthesis of various products are under genic control. They induced mutations to provide a large number of mutants different from the wild type, thus utilizing the technique of mutagenesis discussed in Chapter 17. They also used the microorganism *Neurospora*, whose life cycle was well known. Wild-type *Neurospora* has few nutritional requirements. They are:

1. Certain inorganic salts.
2. A source of carbohydrate.
3. The vitamin "biotin."

Since these three are absolutely essential, they must be supplied in a "minimal medium" that provide the only basic requirements for wild-type *Neurospora*. If these three are supplied, the organism is capable of producing the many vitamins, amino acids, and other organic requirements necessary for growth.

The original experimental procedure of Beadle and Tatum involved the use of X rays to produce mutations. They let the mutants mate with a wild type and then looked for abnormal types in the segregating generation. If a mutant failed to grow on the minimal medium, it was assumed that there was a "genetic block" in the synthesis of some biochemical compound necessary for the life and growth of the organism. If the missing metabolite were supplied and growth ensued, the missing metabolite could be identified empirically by providing, in turn, various metabolites to the culture. This is a simplification of an extremely complicated and ingenious technique. For this and other researches Beadle and Tatum, together with Joshua Lederberg, were awarded the Nobel Prize in Medicine for 1958.

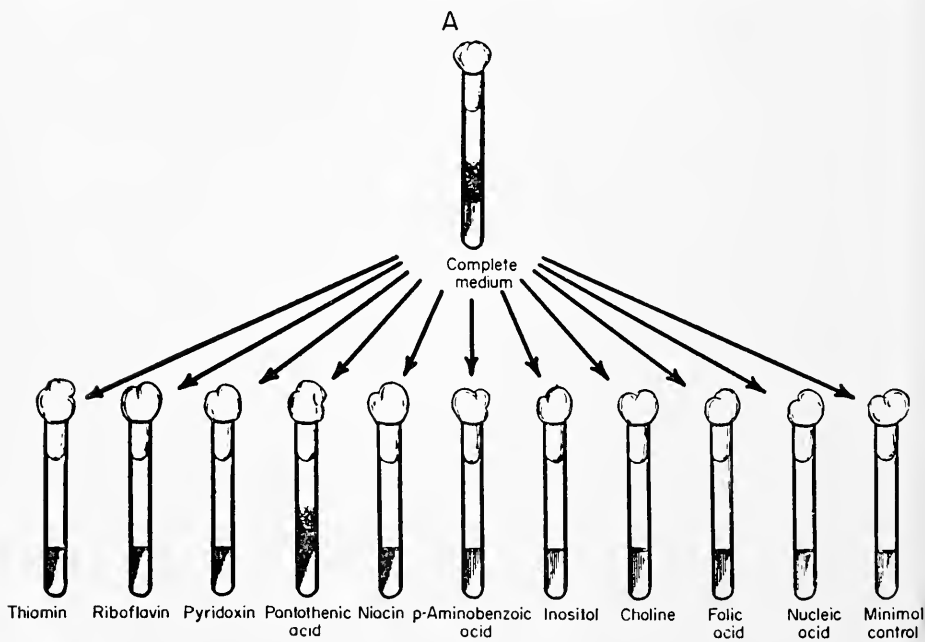
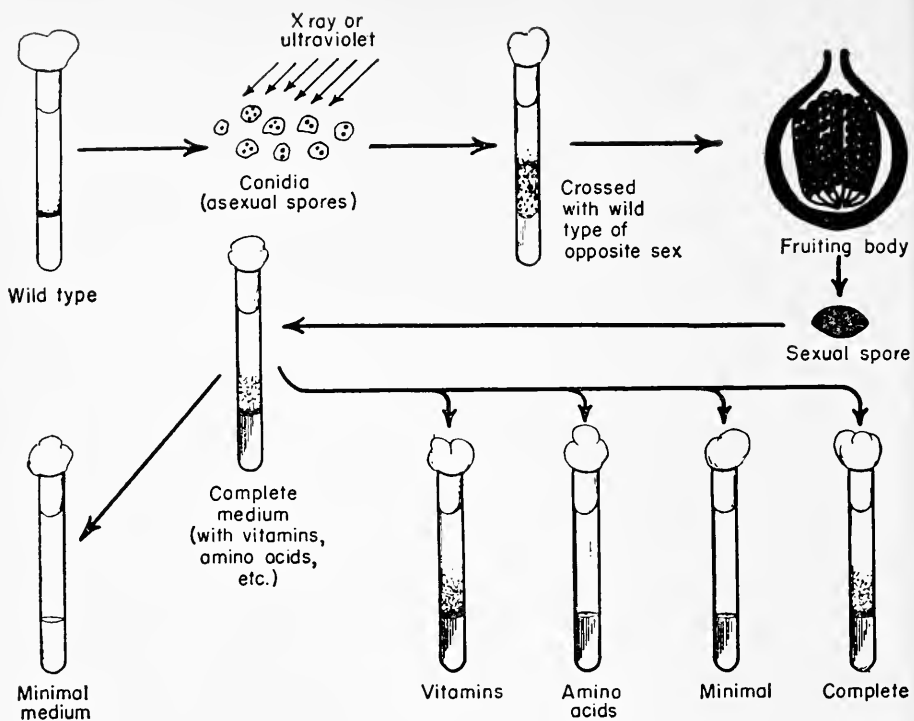
One of the reasons why *Neurospora* is such a good genetic tool is that *all four* of the products of a single meiosis can be observed directly. These all divide equationally, so that an ascus has eight ascospores. Following the induction of the mutant and the mating with wild type, a single sexual spore is isolated from the segregation resulting from meiosis. This spore is then grown on a complete medium to insure the increase of the mutant for further testing. Asexual spores (or mycelia) from this culture are then used to inoculate test tubes containing different media (Fig. 18-4).

In this example it is observed that growth takes place on the complete medium and on the minimal medium fortified with vitamins. But no growth occurs on the minimal medium or on the medium lacking any amino acid. From these tests it is obvious that the mutant lacks the ability to synthesize some amino acid. Further testing is necessary to determine which amino acid is involved. This can be done by providing a number of test tubes supplied with minimal medium, but each fortified with a different amino acid. Suppose, for example, the mutant does not grow in minimal medium fortified with arginine, leucine, or lysine, but does grow in a minimal medium plus methionine. It would be obvious that the induced mutant lacked the ability to synthesize methionine.

By similar techniques and rather laborious trials, a large number of mutants lacking the ability to synthesize a variety of vitamins, amino acids, and other compounds have been produced.

MASS PRODUCTION OF NEUROSPORA MUTANTS

A rather ingenious technique for obtaining *Neurospora* mutants in greater numbers has been devised by Val Woodward, *et al.* (1954). This is illustrated in Fig. 18-5. The asexual spores are irradiated, as in the technique



B

Courtesy of G. W. Beadle; and Yale University Press

FIG. 18-4 Method of detecting induced mutants in *Neurospora*. (a) Mutant identified as lacking in ability to synthesize some vitamin. (b) Individual vitamin identified (pantothenic acid).

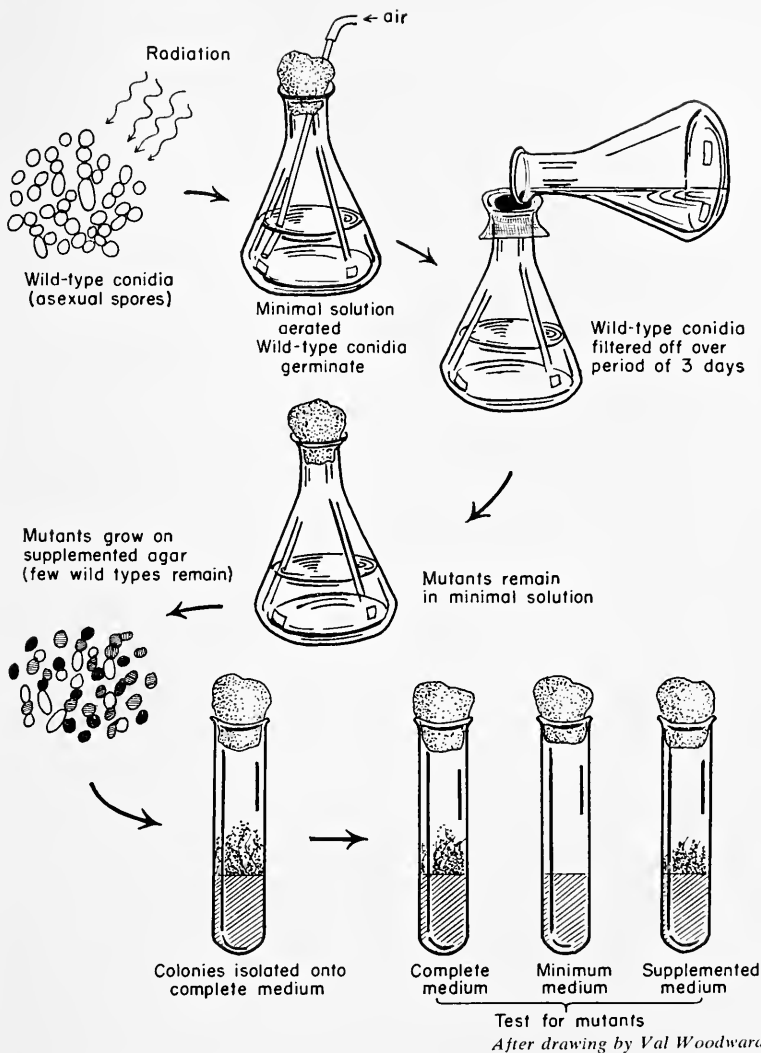


FIG. 18-5 Mass production of mutants in *Neurospora*. Conidia allowed to germinate in minimal liquid medium. Wild-type spores send out hyphae which become entangled in filter through which liquid medium is strained. This leaves high proportion of ungerminated mutant conidia, which are later tested to detect mutants induced.

just described. The improvement in the Woodward technique consists in growing the irradiated spores in a liquid minimal medium, where the non-mutant spores start to germinate, sending out mycelia. These mycelia become entangled in the filter through which the culture is poured periodically over a three-day period. At the end of that time the culture liquid contains a fairly high percentage of mutants that have been unable to germinate in the minimal

medium. These are then permitted to grow on a complete medium, and individual mutants are tested to determine the genetic constitution of the mutants as described previously.

STEPWISE SYNTHESIS OF BIOCHEMICAL PRODUCTS

The many biochemical compounds synthesized in a growing and developing organism are synthesized in a stepwise manner. It is, in a way, comparable to the steps in the stairs leading from a lower to a higher level. If these stairs are unobstructed, traffic can proceed from the bottom to the top, but if a bar is put across any step, access is stopped to the steps above.

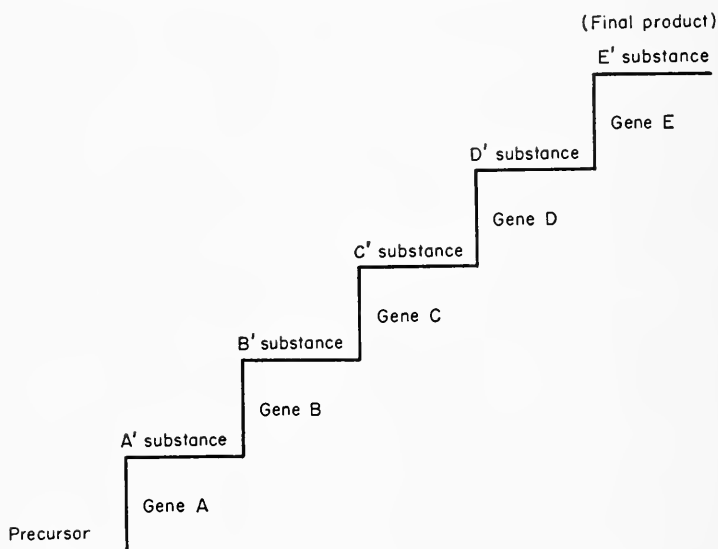


FIG. 18-6 Stepwise action of biochemical mutants. Recessive (mutant) allele blocks formation of all higher products.

It makes no difference where this bar is placed, as far as final access to the top is concerned (Fig. 18-6). However, it does make a difference as far as the intermediate products of synthesis are concerned. If the "block" is caused by a recessive gene's failing to supply any enzyme, for example by recessive gene *a*, then all substances from A' on are not produced. However, a block by a recessive gene *e* would prevent the formation of only the final product. The analogy of the stairs emphasizes the step-wise production of the biochemical products.

GENETIC BLOCKS CAUSE ACCUMULATION OF INTERMEDIATE PRODUCTS

Obviously, a genetic block at any intermediate point will result in a "piling up" of the metabolic products that result from carrying out the

biosynthesis of steps before the one blocked. A stoppage at step D on the hypothetical stairs would result in a piling up of substance C'. Due to a recessive gene *d*, it is not possible to convert substance C' to D', because some essential enzyme is lacking.

In a wide variety of organisms it is now perfectly clear that a genetic block leads to the accumulation of metabolic products laid down before the genetic block, or on a lower stair, to use the analogy of the stairway. By studying the products accumulated as a result of several genetic blocks in the production of an end product, it is possible to tell the sequence in which the different products appear.

COUNTERACTION OF GENETIC BLOCK BY ADDITION OF BLOCKED SUBSTANCE

To return to the analogy of the stairs, if a recessive gene *c* blocks the production of substance C', then the production of all substances above C' is inhibited. If, however, some of the substance C' is added to the culture medium, then all steps above C may proceed normally. A particularly good illustration of this is in the synthesis of the amino acid methionine in *Neurospora*, which was worked out by N. H. Horowitz at the California Institute of Technology in 1947. He found that two genes were involved in the conversion of cysteine to homocysteine and thence to methionine.

To return to the analogy of the stair steps, the action proceeds as shown in Fig. 18-7. The wild-type allele of gene 1 is necessary for the conversion of

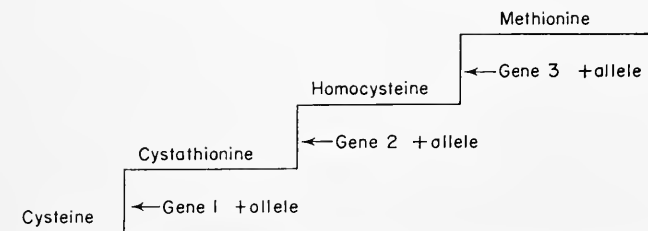


FIG. 18-7 Methionine metabolism in *Neurospora*. Plus allele of genes 1, 2, and 3 necessary to supply enzymes that enable reaction to take place.

cysteine to cystathionine. Unless the wild-type allele of gene 2 is also present, the action stops at cystathionine and this substance accumulates in the medium. If the genetic block is at position 1, and gene 1 is recessive, no cystathionine is produced.

It was shown later (see Strauss 1960) that certain mutants will grow on a minimal medium only if methionine has been added. Others will grow if the medium is supplemented with *either* methionine or homocysteine. Still others will grow if the medium has been supplied with any one of the three—

methionine, homocysteine, or cystathionine. By referring to the diagram it is easy to see where the genetic block, conditioned by these three recessive genes, takes place.

GENETIC BLOCKS AND ENZYME ACTIVITY

The most plausible theory for the explanation of the genetic block, conditioned by a single mutant gene, is that the enzyme activity for a particular biochemical reaction has been eliminated or seriously reduced, so that the normal reaction cannot take place. As a result of this inactivity of an enzyme, the precursor or substance involved in the reaction accumulates in the medium, or may be excreted or converted to some other derivative. The genetic block may occur at any one of a number of places along the pathway of the biological synthesis of the end product. The reaction can be stimulated to proceed normally if any one of the products inhibited by the genetic block is supplied to the medium.

The action of enzymes may be altered, or partially or totally inhibited. Since the genetic blocks have this profound effect on enzymatic action it has been postulated that single genes control the activity of specific enzymes, and that when a mutant is produced a specific enzyme is directly affected. This has led to the one gene—one enzyme hypothesis, which states simply that each gene controls the synthesis of, or the activity of, but a single enzyme.

BIOCHEMICAL GENETICS OF BACTERIA

Since the “explosion” of biochemical genetics dating from the *Neurospora* work of Beadle and Tatum (1941), much information has been gained regarding the genetics of bacteria and viruses. One of the leaders in bacterial genetics has been Lederberg, a student of Tatum at Yale University. They discovered that there is recombination of genic materials in bacteria, the same as in higher organisms. Perhaps we should say that the higher organisms are similar in their inheritance to the bacteria and viruses, since in the evolutionary scale the viruses and bacteria came before the higher organisms.



FIG. 18-8 Joshua Lederberg, pioneer in bacterial genetics.

It has been demonstrated by several research workers that mutants lacking ability to synthesize certain metabolites occur in bacteria, the same as in *Neurospora*. Radiation (either X ray or ultraviolet) or certain chemicals are effective in increasing the mutation to these types. The technique used is similar to that used for *Neurospora*.

Bacteria are usually grown on a complete medium which supplies all of the necessary biochemicals for growth. Normal bacteria can synthesize most of the metabolites if grown on a minimal medium. A bacterium widely used in mutagenic studies is *Escherichia coli*, a nonpathogenic type found in the human intestine. From a minimal medium containing glucose and certain inorganic salts, *E. coli* can synthesize all the necessary sugars, phosphates, vitamins, amino acids, and other metabolites necessary for growth.

The normal type is called a *prototroph*, and the mutant type incapable of synthesizing some metabolite is called an *auxotroph*. The colonies are irradiated and allowed to develop on a complete medium, then grown on minimal medium and on media to which different metabolites have been added to the minimal medium. In this manner it has been possible to isolate auxotrophic mutants that lack the ability to synthesize certain vitamins, amino acids, and other compounds, such as purines and pyrimidines. In each case, it has been established that an inability to synthesize some product is due to a single gene, paralleling the behavior in the *Neurospora* mutants.

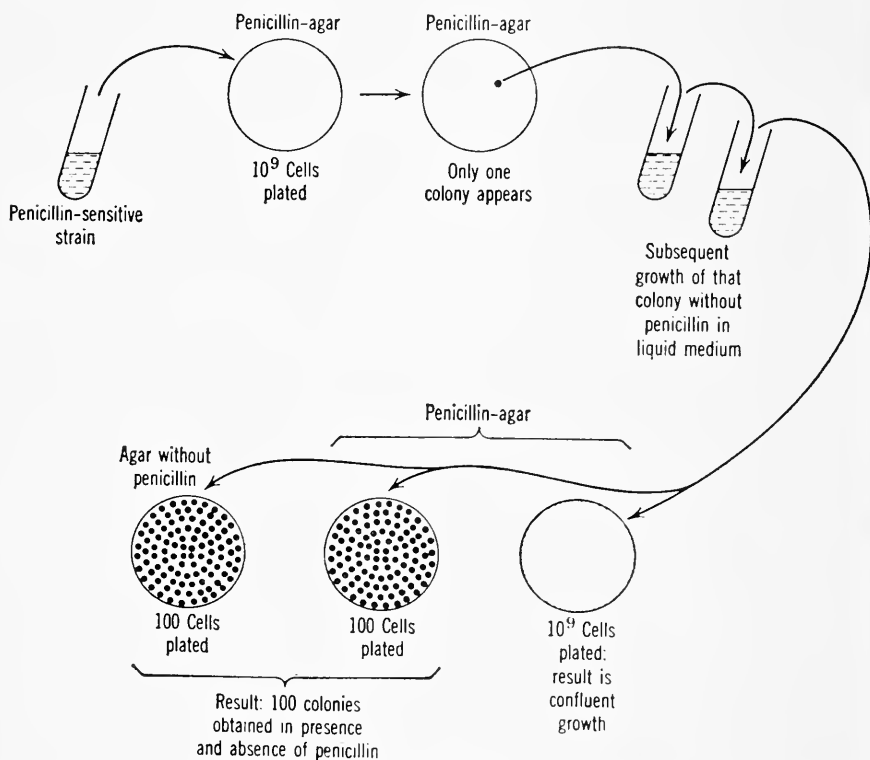
BACTERIAL MUTANTS RESISTANT TO ANTIBIOTICS

Today's world-wide use of antibiotic medicines, which have cured many dread diseases (pneumonia, for example) grew out of the original laboratory work of the British scientist, Sir Alexander Fleming, in the late 1920's. He discovered that the mold penicillium produced a substance toxic to bacteria. However, penicillin as a medicine was not sold until about the time of World War II. Two developments were responsible for its full production. One was an induced mutant having twice the amount of penicillin of the normal. This strain resulted from X irradiation of penicillium and was produced by M. Demerec at the Cold Spring Harbor Biological Laboratory. The second achievement was an improved culture medium, a liquid one, by H. Florey at the United States Department of Agriculture Regional Laboratory in Peoria, Illinois. It was this latter development that put penicillin into business in a big way. Instead of culturing the penicillium on solid media, Florey devised the method of culturing it in corn steep liquor. This substance is a by-product of the wet milling industry of corn kernels, in which starch, glucose, corn oil, and other products are manufactured. Corn steep liquor was available in quantity and proved to be an excellent culture medium for penicillium, giving yields of penicillin four or five times that on solid media.

The prescription of antibiotics really exploded after the war. Almost any ailment was treated with a dose of penicillin. Little was known then about mutations in bacteria and not much was thought about the possibility of selecting bacterial mutants resistant to penicillin. I know one physician who in the earliest years was concerned about the indiscriminate use of the drug. She was Dr. Elizabeth Harrison, our family pediatrician and daughter of Ross Harrison of Yale University. From the beginning, she insisted on no "half-

baked therapy." If she ordered penicillin, she gave large doses and continued until the child was completely recovered.

Dr. Harrison was not a geneticist, but she was following sound genetic principles. It has been established since, in many experiments with different



Courtesy of Ruth Sager and Francis Ryan; and John Wiley and Company

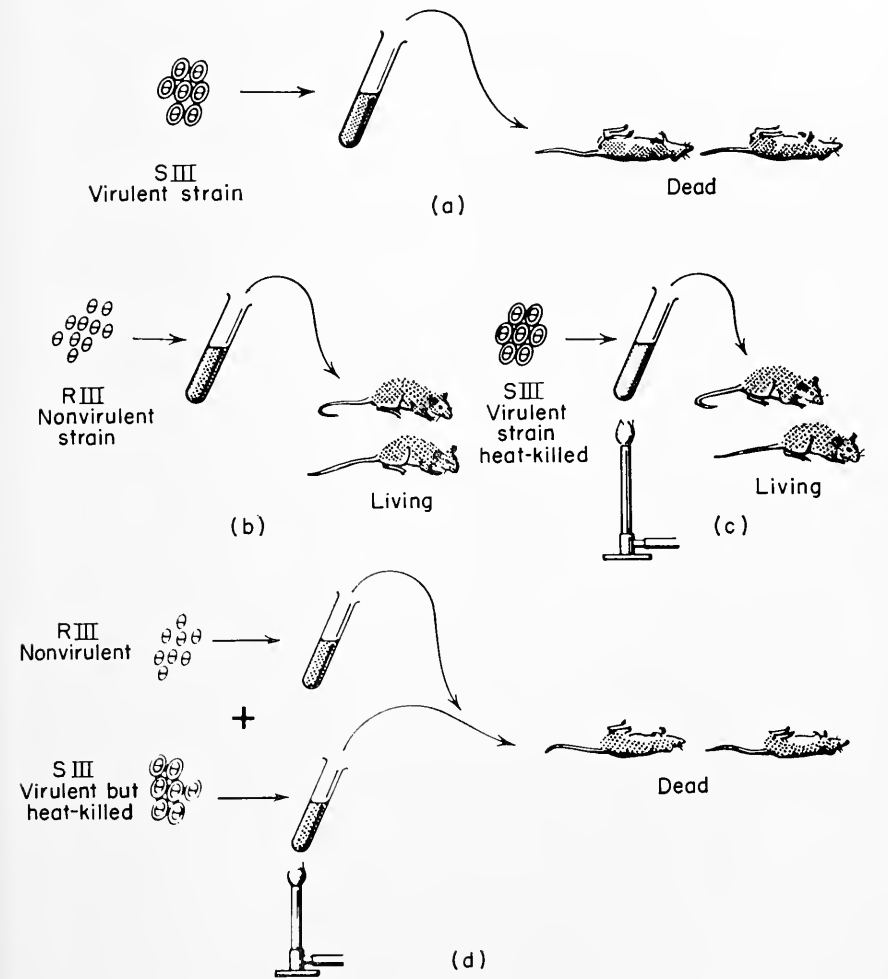
FIG. 18-9 Selection of a drug-resistant strain of bacteria. To select a drug-resistant strain, a heavy suspension of drug-sensitive cells is placed on agar containing the drug. Most cells die, and any colonies that appear are probably drug-resistant. They are further tested by growth for several generations in liquid culture in the absence of the drug, followed by plating again on drug-containing agar. If the strain is now resistant, a colony should form from every cell, both in the presence and in the absence of the drug. If a heavy suspension (e.g., 10⁹ cells) of drug-resistant cells is plated on drug-containing agar, the growth will be confluent. The suspension must be diluted so that only about 100 cells are plated; the resulting growth will be in the form of discrete countable colonies.

organisms, especially *E. coli*, that mutants resistant to an antibiotic may be selected following the application of not quite lethal amounts (Fig. 18-9). An insufficient dose does not produce the mutants. Instead, by killing nearly all of the susceptible bacteria in the colony, it allows the resistant ones to develop. From the surviving, it is possible to select more resistant types of

bacteria by a repetition of the process. The implications of these resistant types to the health of man will be discussed in Chapter 19.

TRANSFORMATION OF GENETIC MATERIAL

Transformation is a novel way of changing genetic material from one genotype into another. This is not done by the conventional method of



Courtesy of Ruth Sager and Francis Ryan; and John Wiley and Company

FIG. 18-10 Transformation in bacteria. Diagram of Griffith's experiment. (a) Mice injected with a virulent strain of pneumococcus, SIII, die of pneumonia. (b) Mice do not die when injected with a non-virulent strain, RII, which does not possess a capsule but comes from a virulent, encapsulated strain SII. (c) Mice do not die when injected with a virulent strain, SIII, which has been heat-killed. (d) When mice are injected with a mixture of heat-killed SIII and non-virulent RII, they die of bacteremia, and living SIII organisms are found in their bodies.

crossing and then looking for recombinants in a segregating generation. Instead a portion of the genetic material is changed by growing one type of bacteria in the presence of an extract of another.

Transformation was discovered in 1928 when Griffith in England, injecting mice with different strains of pneumococcus, made a remarkable finding. He injected mice with two strains of the bacteria, one a nonvirulent form and the other a virulent form that had been killed by heat. Either of these injected by itself into mice produced no effect. However, when they were both injected into the same mouse, the animal died. In some way the nonvirulent form was *transformed* into a virulent strain that killed the mouse. For a complete description of this exciting work, the student is referred to *Cell Heredity* by Sagar and Ryan (1961). Two illustrations of the operation of this technique are reproduced here (Figs. 18-10 and 18-11).

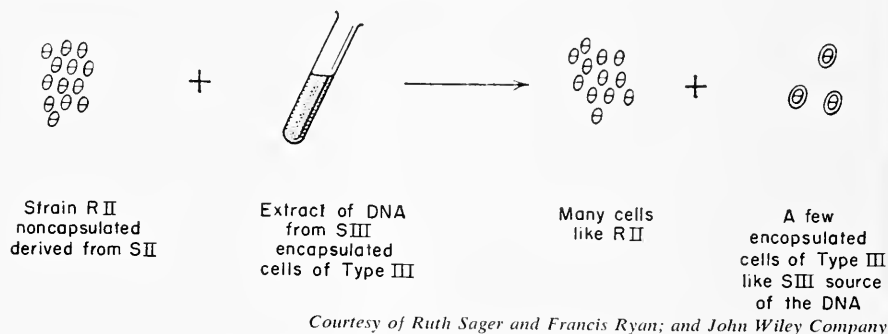


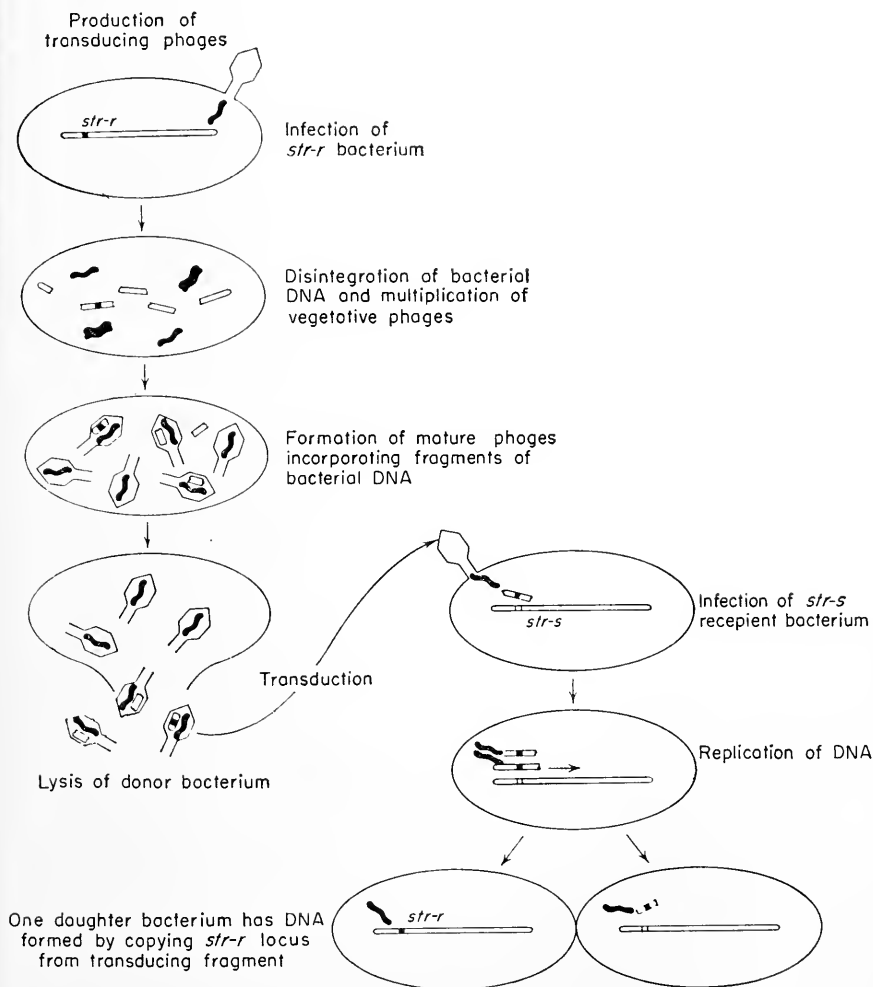
FIG. 18-11 Transformation of capsular type in pneumococcus. Cells of strain RII, which are nonencapsulated but derived from encapsulated strain SII are treated with an extract of DNA from encapsulated cells of strain SIII. After treatment, many cells are recovered like the parental strain RII but, in addition, some encapsulated cells of Type III are found like strain SIII which was the source of the DNA. These cells are *transformants*, having acquired from the DNA extract new genetic material for making Type III capsules.

Apparently, virulence in this organism is dependent upon the presence of a polysaccharide capsule around the cells. The new, transformed bacteria were encapsulated just like the virulent strain that had been killed by heat. This encapsulation process has been performed *in vitro* as illustrated in Fig. 18-11. The transformed bacteria remain encapsulated. They and their extracts also had the same transforming ability as did the original virulent strains, and extracts from them. The extracts have been analyzed and shown to be deoxyribonucleic acid (DNA).

TRANSDUCTION OF GENETIC MATERIAL

Transduction is the process of the transfer of genetic material from one genotype to another. In this case the transfer is made by a bacterio-

phage acting as "messenger boy." To quote Sager and Ryan, "When streptomycin-sensitive bacteria, for example, are infected with phages that have been grown on streptomycin-resistant hosts, a fraction (ca. 10^{-6}), of the cells that survive infection become streptomycin-resistant and can thenceforth act as donors of that character" (Fig. 18-12).



Courtesy of Ruth Sager and Francis Ryan; and John Wiley Company

FIG. 18-12 A diagram of the transduction process showing the steps which may be involved.

These phages during their multiplication in the resistant host have in some way incorporated into their genotype some of the DNA from the bacterial host. This DNA is then passed on to the sensitive host and made a part of the genotype.

Thus we see that by transformation and transduction the genotype can be altered just as truly as by conventional means of crossing and recombination.

STREPTOMYCIN-DEPENDENT *E. COLI*

One unusual mutant arose in experiments in which *E. coli* was treated with sublethal doses of the antibiotic streptomycin. Not only were resistant bacterial mutants found, but one actually required limited amounts of streptomycin in the culture medium. If the streptomycin were not added, the culture would die. Thus not only was a bacterium resistant to streptomycin isolated, but it was also a mutant absolutely dependent upon the antibiotic for growth.

CONCLUDING REMARKS

The intensive study of biochemical genetics began with the classic studies of Beadle and Tatum. Using *Neurospora* as genetic material, they induced by irradiation a number of mutants that were unable to synthesize certain metabolites. The inability in each case was inherited as a monogenic recessive. The technique for isolating and testing these mutants is described.

By studying many biochemical mutants, it was learned that certain biological processes are under the control of several genes in a stepwise fashion, each step being under the control of a single gene. The most likely explanation of the phenomenon of mutation is that the recessive gene fails to supply some vital enzyme so that the biological process is interrupted at this point. When a biological process is obstructed, there is an accumulation of the intermediate metabolic products formed before.

The biochemical synthesis of a number of products is discussed, as well as novel ways of transferring or transforming genic material by transduction and transformation.

The isolation of mutants resistant to antibiotics is discussed with implications for their use as medicines. It should be emphasized that all of the genetics discussed in this chapter is haploid genetics, uncomplicated by dominance found in diploid organisms. A study of the microorganisms has contributed markedly to our understanding of genetics and of the nature of the gene.

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PROBLEMS

18-1. Define or describe the following terms:

antibiotic	penicillin
auxotroph	prototroph
biochemical genetics	pyroxidine
corn steep liquor	step-wise synthesis
cystathionine	streptomycin dependent
cysteine	thiamine
deoxyribonucleic acid	transduction
genetic block	transformation
host	transplant (eye)
metabolite	vitamin B6
minimal media for <i>Neurospora</i>	vitamin biotin
non-disjunction	

18-2. Identify the following scientists, giving a major contribution, with an approximate date:

Beadle, G. W.	Horowitz, N. H.
Demerec, M.	Lederberg, J.
Dodge, B. O.	Ryan, Francis
Ephrussi, B.	Sager, Ruth
Fleming, Alexander	Tatum, E. L.
Florey, H.	Woodward, Val
Garrod, A. E.	

- 18-3. In *Drosophila* when cinnabar (*cn*) eye primordia are transplanted into vermilion (*v*) larvae, vermilion eye discs develop. In the reciprocal transplantation when *v* eye primordia are transplanted into *cn* hosts, the eye which develops is the wild type. Show by a diagram the order in which the dominant alleles *v*⁺ and *cn*⁺ act to produce a wild-type eye (fully colored.)
- 18-4. Outline the step used by Beadle and Tatum in their experiments to induce biochemical mutants in *Neurospora*.
- 18-5. How would you design an experiment to isolate a mutant for streptomycin resistance in *E. coli*?
- 18-6. Fig. 18-9 illustrates the selection of a penicillin-resistant strain of bacterium. Was this resistant mutant produced by the penicillin agar on which it was grown? Explain.
- 18-7. Many genes have been discovered that affect synthesis of certain compounds in *Neurospora*. None of the mutants will grow on minimal medium. The

following table shows either growth or no growth when different substances are added.

Gene	Minimal medium plus substance below	Growth +, no growth -
a^+	arginine	+
	citrulline	-
	ornithine	-
b^+	arginine	+
	citrulline	+
	ornithine	-
c^+	arginine	+
	citrulline	+
	ornithine	+

What is the order of synthesis of these three products? Show by a diagram where genes a , b , and c , produce genetic blocks.

Biochemical Genetics in Man

"OF INBORN ERRORS of metabolism, alkaptonuria is that of which we know the most, and from the study of which most has been learnt." *Alkaptonuria*, A. E. Garrod (1908).

The lines above, quoted from the opening of Garrod's Croonian Lecture on Alkaptonuria, are as true today as when written, though this fact cannot be confirmed by casual reading of the available texts. The usual sources present only a restricted view of what has been a rich and many-faceted problem, and one which in turn is still enriching our understanding of biological processes. The disease was the prototype of the inborn error of metabolism. It was the first hereditary disease whose mode of transmission was known, and through its use the first intermediary metabolic pathway was elucidated. (W. E. Knox (1958).)



Courtesy of Dr. Victor A. McKusick,
The Johns Hopkins Hospital;
and C. V. Mosby Co.

FIG. 19-1 Sir A. E. Garrod, father of biochemical genetics.

The foregoing quotation of Garrod in 1908, and the excerpt from Knox just half a century later, were taken from a research paper by Knox published in Vol. 10 of the *American Journal of Human Genetics*. A photograph of Dr. Garrod, published in the same journal, appears as Fig. 19-1.

The inheritance and the metabolic nature of this disease were deciphered by Garrod as early as 1902. In 1908 he presented in a lecture nearly all the facts that we know today concerning it.

The different enzymes were postulated, but not identified. Six enzymes necessary for the different steps in the catabolism of phenylalanine and tyrosine to acetoacetic acid are listed by Knox (1958). The acetoacetic acid produces CO_2 and H_2O , the end products in the normal degradation of phenylalanine and tyrosine.

The most striking manifestation of alkaptonuria is the excretion of urine that turns dark to black upon exposure to air. The excretion of the unusual substance in the urine is due to a genetic block in the normal metabolism of tyrosine. This occurs in persons who are homozygous for the recessive gene. Homozygous recessive persons lack the enzyme *homogentisate oxidase*, which is necessary to convert homogentisic acid to maleylacetoacetic acid, the next step in the degradation process. Since this enzyme is lacking, there is no conversion of homogentisic acid, hence an accumulation of this product, which is excreted in the urine. Homogentisic acid, also called *alkapton* (hence the name alkaptonuria), is a strong reducing agent, oxidizing readily to form the black pigment.

The block in the degradation may be visualized in a stepwise fashion, similar to that utilized in Chapter 18 (Fig. 18-6), in which the stairs were used as an aid in visualizing the stepwise reaction. In the case of alkaptonuria, we must begin at the top of the stairs and proceed downward, since this is a process in degradation of biochemicals rather than in synthesis (Fig. 19-2).

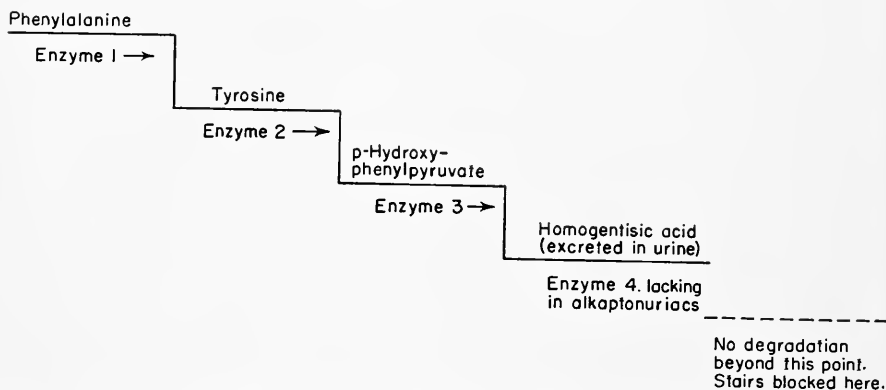
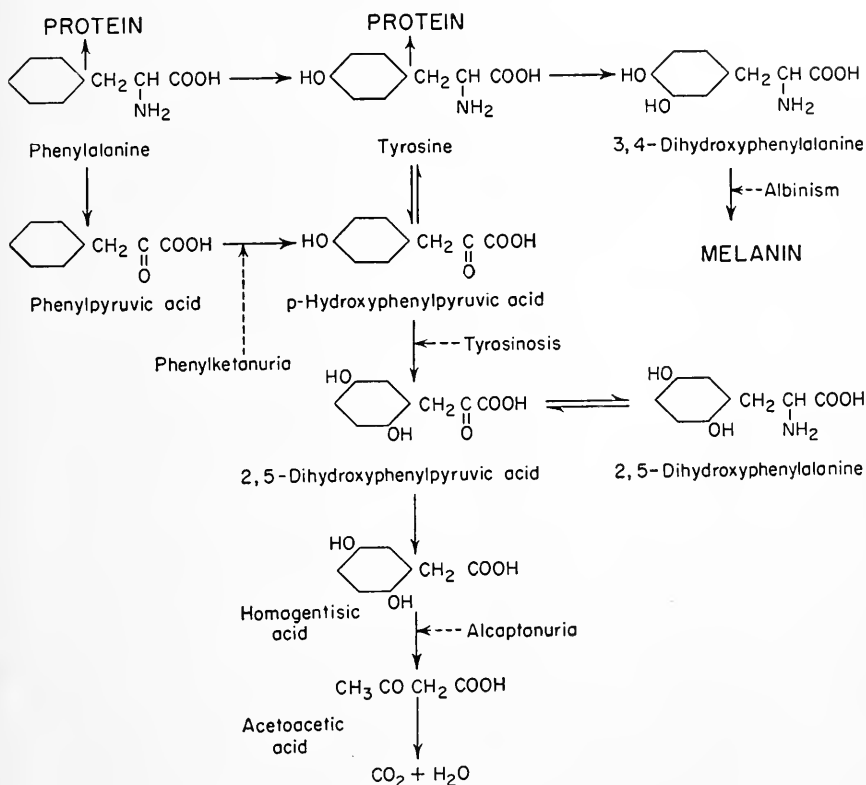


FIG. 19-2 Genetic block in the phenylalanine-tyrosine catabolism, giving rise to disease alkaptonuria.

Since there is no degradation of homogentisic acid, it accumulates in alkaptonurics and is excreted in the urine.

Garrod found maximal excretion of homogentisic acid to occur four to seven hours after a meal, coincident with the normal excretion of nitrogen. From this he concluded that tyrosine from the diet was absorbed and converted into homogentisic acid. He also cited examples, in his 1908 lecture, before the Royal College of Physicians in London, of cases where the feeding

of tyrosine to alkaptonurias resulted in increased excretion of homogentisic acid. Likewise, when homogentisic acid was fed, an increased amount of homogentisic acid was excreted. In normal persons, however, the feeding of tyrosine or homogentisic acid resulted in negative tests in the urine. Such persons had the enzyme to break down the homogentisic acid. The steps in the degradation of phenylalanine and tyrosine are given in Fig. 19-3.



Courtesy of G. W. Beadle and Chemical Reviews.

FIG. 19-3 Biochemistry of the degradation of phenalanine. Alkaptonurias are homozygous for a recessive gene that blocks step shown. Homogentisic acid is secreted in the urine. Genetic block in another place in phenalanine catabolism produces albinos (no melanin).

GARROD'S UNDERSTANDING OF STEPWISE METABOLISM

Although Garrod's work did not immediately have an impact upon the science of genetics, his work is a classic in reasoning and lucid writing regarding the inborn errors of metabolism. Alkaptonuria was the first example. In order that the student may become acquainted with the writing of this man,

who was really our first biochemical geneticist, portions of a few paragraphs taken from Garrod's 1908 address are reproduced below.

The conception of metabolism in block is giving way to that of metabolism in compartments. The view is daily gaining ground that each successive step in building up and breaking down, not merely of proteins, carbohydrates, and fats in general, but even of individual fractions of proteins and of individual sugars, is the work of special enzymes set apart for each particular purpose.

It may be that the intermediate products formed after several stages have only momentary existence as such, being subjected to further change almost as soon as they are formed; and that the course of metabolism along any particular path should be pictured as in continuous movement rather than as series of distinct steps. If any one step in the process fails the intermediate product in being at the point of arrest will escape further change, just as when the film of a biograph is brought to a standstill the moving figures are left foot in air. All that is known of a course of catabolism tends to show that in such circumstances the intermediate product in being is wont to be excreted as such, rather than it is further dealt with along abnormal lines. Indeed, it is an arguable question whether, under abnormal conditions, the metabolic processes are ever thrown out of the ordinary lines into entirely fresh paths, with the results that products are formed which have no place in the normal body chemistry.

It is commonly assumed that this happens, but if the conception of metabolism in compartments, under the influence of enzymes, be a correct one, it is unlikely, *a priori*, that alternate paths are provided which may be followed when for any reason the normal paths are blocked. It is far easier to suppose that in such circumstances normal intermediate products are excreted without further change and that processes which in health play but small parts in metabolism are called into unwonted activity.

When an endeavor is made to classify the unusual constituents which are occasionally present in that most important animal excretion, the urine, it is found that there are few of them which cannot be accounted for as intermediate products, incompletely burnt, or as exaggeration of traces normally present . . . (Garrod 1908, *Lancet*, vol. 2, p. 2.)

One of Garrod's big contributions was the realization that the production of a substance like alkapton was the result of no abnormal process or decomposition of material in the intestines, but rather an intermediate step in the *normal* catabolism of tyrosine and phenylalanine. Another big contribution was the understanding that this condition was due to a recessive gene, and not an infectious agent. His view of the heredity of alkaptonuria was presented in his 1902 paper, quite soon after the rediscovery of Mendel's law. Garrod was familiar with the work of Bateson, the eminent British geneticist who had just published a book in 1902, *The Principles of Heredity*. Undoubtedly Garrod's acquaintance with Bateson and his work was a factor in Garrod's understanding of the genetic nature of the disease.

In his 1908 lecture (*Lancet* 1908, vol. 2, p. 5), Garrod presents his ideas regarding the genetic nature of the disease, showing a mastery of the new science of genetics and a comprehension of the genetic nature of alkaptonuria. The following quotation is from his 1908 lecture:

It was pointed out by Bateson, and has recently been emphasized by Punnett, that the mode of incidence of alkaptonuria finds a ready explanation if the anomaly in question be regarded as a rare recessive character in the Mendelian sense. [The rest of the paragraph gives concise and correct interpretation of Mendel's law. Garrod stated in the case of rare recessives, two heterozygotes mate most infrequently. W.R.S.]

When, however, intermarriage occurs between two members of such a family, the chance [of alkaptonuria] will be much greater, and of the offspring of such a marriage, several are likely to exhibit the peculiarity. The rarer the anomaly, the more conspicuous should be the influence of consanguinity.

The most striking phenotypic expression of alkaptonuria is the excretion in the urine of homogentisic acid, a strongly reducing substance that upon oxidation to a brown or black polymer, produces the dark color observed. There are, however, other manifestations of the recessive gene producing the metabolic block. A similar color is also produced in certain mesenchymal tissues, which in middle life causes the blackening of cartilages and related tissues. This condition, called *ochronosis*, produces coloration that can sometimes be seen through the skin, and is always observed upon dissection. Some of the tissues degenerate prematurely. Arthritis, the only result of degeneration now generally recognized, is almost inevitable in this disease after middle age, and may be incapacitating, according to Knox (1958). So we see that a single gene in the homozygous condition has various pleiotropic effects, a characteristic common to many genes studied intensely.

Although Garrod's work made no impact then on the young science of genetics, it ranks today as one of the classics of genetic literature. The foundation of biochemical genetics was laid, although the superstructure was not begun until nearly four decades later.

ALBINISM, ANOTHER GENETIC BLOCK

Garrod realized that his interpretation of the nature of alkaptonuria would find general acceptance, if it could be demonstrated that other hereditary conditions had a similar explanation. He turned his attention to albinism, which he realized was also brought about by a homozygous recessive gene. It has been shown since that albinism results from a genetic block in the tyrosine catabolism. Individuals who are *c/c* (the genotype assumed for albinos in a number of species, including man) have a genetic block that prevents the formation of melanin in the same phenylalanine and tyrosine catabolism. Without melanin, no pigment is formed in the skin or hair, giving the snowy white coloration. Also, no pigment is formed in the retina of the eye. This permits the color of the erythrocytes in the blood vessels of the eyes to be visible and results in the characteristic pink eyes associated with this condition. Albino persons are known to be extremely sensitive to strong light and they often wear dark glasses for protection.

The genetics of albinism has been worked out in a number of species. The

wild-type allele is dominant. Apparently one wild-type allele supplies sufficient enzyme for the production of the normal amount of melanin.

The genetic block for melanin is at a different place than alkapton in the metabolism of tyrosine and phenylalanine (Fig. 19-3). The location of the action of the gene causing albinism is shown, as well as the genetic block causing alkaptonuria.

A brief quotation from Garrod (1908) shows a complete understanding of the nature of this defect.

Taking all the known facts into consideration, the theory that what the albino lacks is the power for forming melanin which is normally possessed by certain specialized cells, is that which has most in its favor and is probably the true one. If so, an intracellular enzyme is probably wanting in the subjects of this anomaly, an explanation which, as we shall see later, brings albinism into line with some other inborn metabolic errors, of which a similar explanation is at least a possible one.

Thus his explanation of the genetic block for alkaptonuria was not an isolated case, but seemed to be the general rule for a class of diseases or abnormalities caused by a genetic interruption in a normal metabolic process.

The principle of the stepwise production of metabolic products, proposed by Garrod, has been found applicable to other hereditary conditions in man. In each, the end result is the interruption by a single gene in the normal metabolic pathway. This was Garrod's thesis. The interruption may lead to consequences of greater or less severity. The block in the production of melanin causes perhaps more inconvenience and injury than the excretion of urine that turns black, although the arthritis and degeneration of connective tissues after middle age can do great harm.

If, however, the metabolism is interrupted at another place to prevent the formation of p-hydroxyphenylpyruvic acid from phenylpyruvic acid, the latter accumulates with grave consequences, as described in the following section. (See Fig. 19-4 for place where this gene produces its metabolic block.)

PHENYLKETONURIA

This third and last example of a biochemical mutant in the tyrosine metabolism of man is by far the most serious of the three. (It should be stated that this text does not present a comprehensive survey of biochemical mutants in man. Rather we have chosen a few outstanding cases, which show the principle involved in the inheritance of biochemical mutants, here illustrated by the action of three genes in the metabolism of tyrosine and phenylalanine.)

Phenylketonuria, *PKU* for short, results in the accumulation of phenylpyruvic acid. Much of this is excreted in the urine, but some remaining can result in brain damage so great that children become mentally retarded. They are known as *phenylpyruvic idiots*. A dramatic story of how this disease can

be corrected, if detected early enough, is told in the *Saturday Evening Post* of November 21, 1959, by Ruth and Edward Brecher.

Dr. Willard Centerwall of the College of Medical Evangelists, Los Angeles, designed a test for detecting PKU in very young infants. A ten per cent solution of ferric chloride is applied to the wet diaper of the baby tested. In normal ones a yellow spot appears. In PKU children, however, the spot on the wet diaper is green—evidence of the presence of phenylpyruvic acid. Among such children, about one in 20,000 will soon show brain damage unless the condition is corrected. The treatment utilizes a principle elucidated by Garrod in his excellent early work. He found in the case of alkaptonuria that increased feeding of foods containing tyrosine caused increased excretion of homogentisic acid. Likewise, a reduction in the amount of such foods consumed decreased the excretion of this substance.

Richard Block, of the Boyce Thompson Institute, Yonkers, New York, found that much of the phenylalanine could be filtered out of foods by activated carbon. He proposed in 1939 that PKU babies be fed a diet in which the phenylalanine had been removed in this way. Lack of interest on the part of physicians, and lack of an easy method of identification of such infants, caused the delay of some 12 years in the testing of the method. Trial was first found to be successful on a two-year-old girl with marked symptoms of the disease. Within a matter of months she was much improved. Addition of phenylalanine to her diet caused a severe relapse, convincing the doctors that this substance produced the effect. Return to a phenylalanine-free diet caused a marked improvement.

Children with PKU are given a severely restricted diet. Foods high in proteins, such as meat and eggs, must be eliminated. Patients are allowed some fruits and vegetables. Limited quantities of milk, enough to provide only a specified amount of phenylalanine, are allowed. A special protein, in which most of the phenylalanine has been removed, supplies the protein needed for body-building.

These findings in regard to children with phenylketonuria illustrate well that this disease is due to a metabolic block in the phenylalanine metabolism. They also illustrate the point that reducing the intake of the precursor, phenylalanine, materially reduces the amount of phenylpyruvic acid produced in the patient. It is the accumulation of phenylpyruvic acid that damages the brain. The injury can be eliminated or substantially reduced by feeding a diet restricted in phenylalanine. It is not known how long this diet must be continued.

PLEIOTROPIC EFFECTS OF PHENYLKETONURIA

In addition to the brain damage suffered by children with this disease, it is also observed that such children are excessively blond, with practically no melanin in their hair. This is understandable if we consider the place where the genetic block occurs—immediately before the production of

p-hydroxyphenylpyruvic acid, a precursor in one of the biological pathways to tyrosine, which is an intermediate step in the production of melanin. Thus the interruption of the metabolism of phenylalanine by the gene responsible for phenylketonuria causes a lowered production of melanin as well as the severe brain damage. These occur in addition to the excretion of abnormally large amounts of phenylpyruvic acid in the urine. Here is but another excellent example of pleiotropy, or the multiple effects of a single gene.

GOUT

Gout is a disease whose presence has been known for many centuries, although it is not understood as completely as alkaptonuria. The symptoms are an elevation in the uric acid content of the blood (*hyperuricemia*) and excessive pain in any one of a number of joints, usually in the lower extremities, particularly the foot or the ankle. In severe cases, deposition of uric acid crystals may occur in the joints, or even in the lobes of the ears. The reason for the hyperuricemia is not as clear as that for the excretion of homogentisic acid in the case of alkaptonuria. It is known, however, that limitations in the amount of purines consumed ordinarily will prevent acute attacks of gout or prolong the period between. The disease is thought to be inherited as an autosomal dominant with greater expressivity in males than in females.

One of the ways of correcting gout symptoms is to prescribe colchicine, either daily as a prophylactic or in large amounts for acute attacks. Colchicine has been used for treatment of gout since the time of the Pharaohs in Egypt. However, it is not certain how the drug acts in prevention, or in alleviating the symptoms, whose severity is almost unbelievable to a normal person. Conceivably a recessive gene blocks the production of an enzyme, whose function is aided by the very small amount of colchicine administered. The amount re-

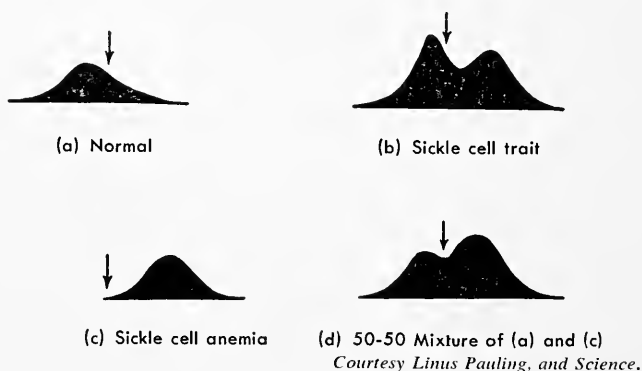


FIG. 19-4 Electrophoretic differentiation of normal hemoglobin and hemoglobin S in h^a/h^a , normal (a); h^s/h^s sickle cell trait (b); sickle cell anemia h^a/h^s (c); and a mixture of (a) and (b).

quired for a prophylactic dose is less than one part in 100 million parts body weight of a normal man. To alleviate acute attacks it is necessary to give approximately five times this amount.

GENES AND HEMOGLOBIN IN MAN

Hemoglobin is the substance that gives the color to red blood cells. There are a great many molecules of hemoglobin ($ca. 2.8 \times 10^8$) in one red blood cell.

A type of anemia in the Negro race has been found to be caused by an abnormal hemoglobin (hemoglobin S). Linus Pauling *et al.* (1949) found that the abnormal hemoglobin molecule can be separated electrophoretically from the normal (Fig. 19-4). The chemical differences between the abnormal and the normal are slight. According to Leavell and Thorup (1960), of the nearly 300 amino acids present in each of the identical half molecules of hemoglobin, only one is abnormal in hemoglobin S. One normally occurring glutamic acid is replaced by valine, with the net loss of two carboxyl groups by the entire molecule.

From a genetic standpoint the interesting thing about this anomaly is that a specific gene causes the production of hemoglobin S. This gene has been designated in various ways. In the one used here, the gene for normal hemoglobin is labeled h^a using the letter h for hemoglobin and a superscript a designating the normal type. The allele for hemoglobin S is h^s . The s in the superscript indicates sickling, since it has been observed that red blood cells of either h^a/h^s or h^s/h^s persons will assume the shape of a sickle or scythe when the oxygen in the blood sample has been reduced sufficiently.

The phenotypic manifestations of the h^s allele are several. Homozygous h^s/h^s persons have a severe disease, called *sickle cell anemia*. Also there is a retarded physical development and an unusual elongation of the arms and legs (Fig. 19-5). Such individuals rarely live to maturity. Heterozygous h^a/h^s persons (*sickle cell trait*) show none of these extreme symptoms, and the presence of the h^s allele must be detected in blood samples. The blood of homozygous h^s/h^s persons may show some sickle-shaped red cells in freshly drawn blood samples, and all of the cells will become sickle-shaped when the oxygen tension is lowered. Red cells of



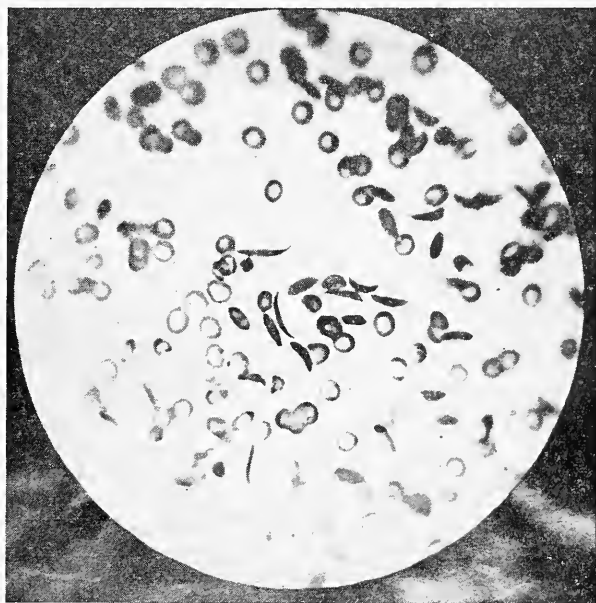
Courtesy of Dr. Byrd S. Leavell, University of Virginia Medical School

FIG. 19-5 Negro homozygous for sickling gene h^s/h^s (sickle cell anemia). Note the extremely long arms and legs, a pleiotropic effect in h^s/h^s persons.

h^a/h^s persons require a more extreme reduction of oxygen to produce the same results.

In summary, the primary effect of the h^s gene is the production of an abnormal molecule of hemoglobin. The sickling, from which the disease gets its name, is a secondary phenomenon.

A blood sample of a homozygous h^s/h^s person is illustrated in Fig. 19-6.



Courtesy of Dr. R. E. Washburn; and Virginia Medical Monthly

FIG. 19-6 Blood cells from homozygous h^s/h^s person. Note sickling of some of the cells. All cells are h^s in genotype. Only those with reduced oxygen show the sickle phenotype.

Some cells are normally disk-shaped in appearance. These all would have been sickle-shaped had the oxygen tension been lowered sufficiently. Since the shape of the cells is rather the result of the amount of oxygen present (or the lack of it), the phenotypic expression of red cells shows an interesting interaction between the heredity (the genotype) and the cellular environment.

Another interesting feature of the sickle cell trait is that persons who are heterozygous h^a/h^s have more resistance to malaria than normal persons h^a/h^a have. Perhaps because of this, the h^s allele has been maintained in greater frequency in the population. Homozygous persons h^s/h^s rarely survive to leave offspring. But since the h^s allele has a selective value in h^a/h^s individuals, there is an explanation for its being maintained with a rather high frequency in the population.

CONCLUDING REMARKS

Biochemical mutants in man (inborn errors in metabolism) have been known almost as long as we have had a science of genetics. As early as 1902 Garrod realized that alkaptonuria was a genetic defect, caused by a block in the normal metabolism of tyrosine. He also was correct in postulating that albinism represented another genetic block. The work of Beadle and Tatum discussed in Chapter 18 added new impetus to the study of genetic metabolic deficiencies. From all the various studies of biochemical mutants have come a series of findings from which might be drawn a number of principles as follows:

1. Normal metabolism proceeds in a stepwise fashion.
2. Enzymes are responsible for the individual steps in the metabolic process.
3. Different genes are responsible for the production of different enzymes.
4. One wild-type allele is usually sufficient for the production of the enzyme in question. Dominance is usually complete.
5. The homozygous recessive is unable to produce the enzyme; consequently, a metabolic process is interrupted. In the event of an interruption there is an accumulation of the product formed immediately before.
6. The production of normal hemoglobin molecules is under genic control. A gene causing the substitution of valine for glutamic acid results in an abnormal molecule, hemoglobin S.
7. The production of the intermediate metabolite can be materially increased by feeding of the precursor to the subject with the recessive gene and also by feeding the metabolite in question. Homogentisic acid may be increased in the alkaptonurics by feeding either tyrosine or homogentisic acid.
8. Conversely, the production of the intermediate metabolite, whose conversion to another product has been interrupted, can be materially reduced by curtailing the precursor. For example, uric acid production can be materially reduced in gouty patients by restricting the amount of foods containing purines, a precursor in the formation of uric acid. In a similar manner, curtailment of phenylalanine in the diet of children with phenylketonuria decreases the amount of phenylpyruvic acid formed and helps to alleviate this disease.
9. The action of the different genes to provide different enzymes for specific biochemical reactions, as just outlined in man, lends convincing support to the "one gene—one enzyme" hypothesis proposed by Horowitz (1950). This theory states that "a large class of genes exist in which each gene controls synthesis of, or the activity of, but a single enzyme."

10. The genetic block causing an interruption in a metabolic process in man has been confirmed in *Neurospora* and other microorganisms. The first case, alkaptonuria, is one of many. All adhere to the same general rule, that genes control specific enzymes necessary for the many steps being activated.

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PROBLEMS

- 19-1. Define or describe the following terms:

albinism	ochronosis
alkapton	phenylalanine
alkaptonuria	phenylketonuria
colchicine	phenylpyruvic idiot
gout	pleiotropic effects
homogentisic acid	sickle cell anemia
inborn error of metabolism	sickle cell trait
melanin	tyrosine

- 19-2. Identify the following scientists, giving a major contribution with the approximate date:

Bateson, William	
Block, Richard	Garrod, A. E.
Centerwall, Willard	Knox, W. E.

- 19-3. How is the inheritance of alkaptonuria similar to that of cinnabar eye color in *Drosophila*?
- 19-4. What are the similarities between the inheritance of alkaptonuria and albinism?
- 19-5. Of what evolutionary significance has the gene for sickling h^s of the red blood cells in man?

- 19-6. The mutation for albinism has occurred in many animals, including man. What is the biochemical nature of this mutant?
- 19-7. Show by a diagram in metabolism where the gene for albinism produces its effect.
- 19-8. Show by a diagram where the gene for alkaptonuria produces its effect.
- 19-9. How is the inheritance of errors of metabolism similar to the biochemical mutants in *Neurospora*?

Population Genetics, or Heredity in Populations

POPULATION GENETICS is a study of the frequencies of genes in a population. Up to now we have been concerned with controlled matings in which two homozygous strains were crossed. If a strain homozygous for gene A , i.e., A/A , is crossed with a/a , the F_1 is A/a and obviously the frequency of gene A equals that of gene a , each being one half. The total frequencies of genes A and a equal $\frac{1}{2} + \frac{1}{2}$, or 1. The F_1 produces two kinds of gametes, each with a frequency of one half, giving the familiar ratio in the F_2 of 1 A/A : 2 A/a : 1 a/a . This is obtained by squaring the proportion of gametes produced in the F_1 ($\frac{1}{2} A + \frac{1}{2} a$)² = $\frac{1}{4} A/A$, $\frac{1}{2} A/a$, and $\frac{1}{4} a/a$. By the same method we obtain $a^2 + 2ab + b^2$ from the expression $(a + b)^2$.

That the proportion of each allele is one half can be demonstrated in another manner (Table 20-1).

Table 20-1. Proportion of A and a Alleles in a Controlled Mating of $A/A \times a/a$

	Genotype	Genes		Proportion of Each
P	A/A	2 A		all A
	\times a/a	2 a		all a
F_1	A/a	A ,	a	$\frac{1}{2} A : \frac{1}{2} a$
F_2	1 A/A	2 A	0 a	$\frac{1}{4} A$ 0 a
	2 A/a	2 A	2 a	$\frac{1}{4} A$ $\frac{1}{4} a$
	1 a/a	0 A	2 a	0 A $\frac{1}{4} a$
Total F_2		4 A	4 a	$\frac{1}{2} A : \frac{1}{2} a$

If each F_2 individual is equally fertile and leaves the same number of progeny, it can be demonstrated easily that the proportion of genes A and a in a population produced by random mating among the F_2 individuals will remain unchanged, one half A and one half a .

The same rule applies to all populations mating at random, including human populations. In these the gene frequencies may differ tremendously from the population of one half to one half in controlled matings between parents homozygous for different alleles of a given gene.

Complete statements regarding the maintenance of the same gene frequencies in a randomly mating population were first made by a British mathematician, G. H. Hardy, and a German physician, Dr. W. Weinberg, simultaneously in independent papers in 1908. They pointed out that gene frequencies would remain constant even though they might not be in the proportion of one half each, as given in the example above for controlled mating of two homozygous types. In fact, the proportion of two genes may be quite different. For example, the gene for albinism (c) in man is much less frequent in any population than the normal allele (C). In some populations the frequency of gene c may be $\frac{1}{100}$ while that of C is $\frac{99}{100}$, and in other populations greater differences may exist. If the frequency of allele c is $\frac{1}{100}$ in a population, the number of albino individuals would be $\frac{1}{100} \times \frac{1}{100}$, or $\frac{1}{10,000}$.

In a population breeding at random, the frequencies tend to remain the same in succeeding generations.

HARDY-WEINBERG LAW

The Hardy-Weinberg law states that in a population mating at random, after one generation of such mating the population is in equilibrium and gene frequencies remain the same. They are in the ratio of $p^2 A/A : 2pq A/a : q^2 a/a$ with $p + q$ always being equal to one. In controlled mating of homozygous types $p = q = \frac{1}{2}$.

In the case of a rare character like albinism (c/c) mentioned earlier, where $p = \frac{99}{100}$ and $q = \frac{1}{100}$ the Hardy-Weinberg formula would tell how many of each genotype would be expected. This is given by the formula:

$$p^2 C/C + 2pq C/c + q^2 c/c$$

Substituting the values of p ($\frac{99}{100}$) and q ($\frac{1}{100}$) in this expression we obtain:

$$(\frac{99}{100})^2 C/C + 2(\frac{99}{100} \times \frac{1}{100}) C/c \text{ and } (\frac{1}{100})^2 c/c$$

From this formula it is possible to determine the relative frequency of the genotypes C/C and C/c . The proportion of the various individuals would be:

$C/C = (\frac{99}{100})^2$	$\frac{9801}{10,000}$
$C/c = 2(\frac{99}{100} \times \frac{1}{100})$	$\frac{198}{10,000}$
$c/c = (\frac{1}{100})^2$	$\frac{1}{10,000}$
Total	$\frac{10,000}{10,000}$

Thus we arrive at a frequency of one albino individual in a population of 10,000. This figure really represented our starting point, because this is the statistic we use in obtaining the frequency of $\frac{1}{100}$ for q in the first place. In any population, randomly mating, it is possible to calculate the frequency of q by taking the square root of the frequency of the observed mutant recessive type in the population. Since, in this hypothetical population, the frequency of albinos is one in 10,000, and since the genotype of an albino is c/c with its probability of q^2 , it is obvious that the frequency c can be obtained from the formula $\sqrt{q^2}$. Substituting, we get $\sqrt{1/10,000} = \frac{1}{100}$. Since $p + q = 1$, $p = 1 - q$ or $\frac{99}{100}$.

The Hardy-Weinberg law enables us to determine the proportion of carriers of a genetic recessive (heterozygotes), to individuals free of the gene for this trait. In the population just cited, the ratio of C/c to C/C individuals is 198:9801, or approximately 1:50. In other words, one of every 51 normal-appearing persons is heterozygous for the gene for albinism. It should be borne in mind that this is a hypothetical case and may not represent the actual proportion of albinos in a population. Also, different populations will not have the same proportion of albinos. The figure chosen, 1/10,000, was selected because of ease of calculation. It is probably too high a rate for various European countries, which have a frequency of 1/20,000. (Stern 1960.) Stern cites ratios of 1/3000 in Negro children in Nigeria and 1/132 among some 20,000 San Blas Indians in Panama. The q value, or frequency of gene c , in these populations would be 1/141 for Europeans, 1/55 for the Nigerian Negroes, and approximately 1/11 for the San Blas Indians. The figure 1/100 for the hypothetical population is within the range of the rates for the different populations.

DETERMINATION OF GENE FREQUENCIES DIRECTLY

In populations in which it is not possible to distinguish homozygotes A/A from A/a phenotypically, it is possible to determine the frequencies of the three different kinds of individuals A/A , A/a , and a/a . This is found indirectly by first taking the square root of the frequency of the a/a class to determine the frequency of q , and then subtracting this value from 1 to give the value of p .

When dominance is lacking, the heterozygote can be distinguished, making it possible to determine *directly* the number of genes in each class, and consequently the gene frequencies. In the Shorthorn breed of cattle, one can make this determination. There are three different colors in this breed: red, roan, and white. The red cattle have two genes for red and the white have two genes for white, while the roan cattle have one gene for red and one for white. There is no dominance. Hence it is misleading to write the gene symbols with a capital and small letter, since the capital letter is conventionally used to indicate dominance.

The genetic designations used in this case are as follows:

R/R = Red Shorthorn
 R/W = Roan Shorthorn
 W/W = White Shorthorn

Sewall Wright (1917) was the first to analyze correctly the inheritance of red, roan, and white Shorthorn cattle and devised the scheme shown above. He presented a summary of the coat color of 8705 Shorthorn cattle of various types of matings. From his summary it is possible to make an analysis of the frequencies of the allele for red (R) and the one for white (W). The red animals would all have two R alleles, the white two W alleles, and the roan one of each. The analysis of the gene frequencies of the 8705 cattle is found in Table 20-2A.

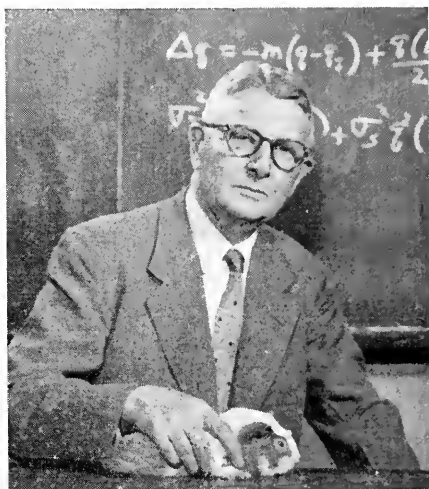


Fig. 20-1 Sewall Wright, who has contributed markedly to the science of population genetics.

In this population the frequency of the R allele (p) is $12,118/17,410 = .696$. The frequency of the W allele (q) is $5292/17,410 = .304$. From these data it is easy to determine whether the population is in equilibrium. The calculated proportions in such a population can be determined from the

Table 20-2A. Gene Frequency Analysis of Coat Color Genes in 8705 Shorthorn Cattle

Color	Number	R Alleles	W Alleles	Total Alleles
Red	4169	8338	0	8338
Roan	3780	3780	3780	7560
White	756	0	1512	1512
Total	8705	12,118	5292	17,410

formula $p^2 + 2pq + q^2$. Substituting the calculated gene frequencies in the formula, we get $.696^2 + 2(.696 \times .304) + .304^2$. This gives the following results:

$$\begin{array}{rcl}
 0.696 \times 0.696 & = & 0.4844 \quad (\text{proportion red cattle}) \\
 2(0.696 \times 0.304) & = & 0.4232 \quad (\text{proportion roan cattle}) \\
 0.304 \times 0.304 & = & 0.0924 \quad (\text{proportion white cattle}) \\
 \hline
 \text{Total} & & 1.0000
 \end{array}$$

Using these calculated proportions of the different kinds of cattle and multiplying by the total number (8705), we get the following:

$$\begin{array}{r}
 46765 \\
 5625 \\
 \hline
 52390
 \end{array}$$

$$\begin{aligned}
 0.4844 \times 8705 &= 4217 \text{ red cattle} \\
 0.4232 \times 8705 &= 3684 \text{ roan cattle} \\
 0.0924 \times 8705 &= 804 \text{ white cattle}
 \end{aligned}$$

Total 8705

Do these calculated numbers of the different colors of cattle differ significantly from the observed numbers? A chi-square calculation gives information on this point. The calculation is shown in Table 20-2B.

Table 20-2B. Genotypes of 8705 Shorthorn Cattle

Color	Observed	Expected	d	d ²	d ² /e
red	4169	4217	-48	2304	0.55
roan	3780	3684	+96	9216	2.50
white	756	804	-48	2304	2.87
Total	8705				5.92

Referring to Table 5-2 in Chapter 5, we see the probability of obtaining a deviation as great as this by chance alone is $< .05$, actually between .05 and .01.

Thus we see a significant deviation from a population mating at random. There is a slight excess of roan animals. The probability value is less than .05, indicating that if we examined 100 such sets of data we would expect to find a deviation as great as this, less than five times out of 100. It is not surprising that such a population should not be in equilibrium because man enters the picture. He can manipulate the breeding of the various animals and he may have been mating more reds to whites than would be expected in a randomly mating population.

Whenever it is possible to differentiate genotypes A/A and A/a and controlled matings are made, there is always a chance for selection. In such a case the mating would not be at random. Completely random mating is more likely to occur when it is *not* possible to detect visually the different genotypes A/A , A/a , or a/a .

RANDOM MATING WITH NO VISIBLE DIFFERENCE IN PHENOTYPE

We might expect to find random mating the rule in populations in which the different genotypes produce no visible difference in phenotypes and where more or less elaborate tests are necessary to determine the genotype. This is true of the blood groups, discussed in Chapter 14. In the case of the MN series of blood groups it is possible, by tests, to distinguish M, MN, and N individuals. Since these blood groups have no outward pheno-

typic expression, no opportunity is offered for selection in mating, and random mating exists.

The frequencies of the M and N alleles differ greatly between different populations, but any population should follow the Hardy-Weinberg Law regarding the distribution of the different genotypes. The gene frequencies of M and N can be determined directly, as in the case of the Shorthorn cattle.

Two different human populations are chosen in this text for illustrative purposes, with widely different frequencies of the M and N genes (Table 20-3). (The data are from Boyd.) The gene frequencies given in Table 20-3

Table 20-3. Frequencies of Genes M and N in Two Vastly Different Populations

Population	No.	Genotypes			Gene Frequencies		Genotypes Expected			
		M/M	M/N	N/N	$p(M)$	$q(N)$	$p^2 M/M$	$2pq M/N$	$q^2 N/N$	
Australian Aborigines	102	3	44	55	0.25	0.75	6	38	58	
American Indian (Pueblo)	140	83	46	11	0.76	0.24	81	51	8	

may be calculated directly in the same manner as the gene frequencies for red, roan, and white in Shorthorn cattle. M/M individuals have two M genes and N/N individuals two N genes, while M/N persons have one M and one N gene. It is also possible to calculate the expected number of individuals with the different genotypes, once the frequency of M and N has been obtained. The chi-square comparison of the observed and expected reveals no great deviation of the observed from the expected ratios. The probability values are roughly .3 and .5, showing no significant departure from the expected.

What do the figures mean? The frequencies of M and N are practically reversed, yet in both cases the frequencies of M and N follow the Hardy-Weinberg formula $p^2 M/M + 2pq M/N + q^2 N/N$. The values are completely different from those for a controlled mating where $p = q = \frac{1}{2}$.

These figures illustrate that the value of p and q may vary tremendously and yet follow the Hardy-Weinberg formula, showing that the population is in equilibrium and mating at random. How the two populations become so different is another and very interesting story.

Two of the main factors that may have been responsible for the change are selection and genetic drift. The subject of selection will be considered more extensively in Chapter 21 on evolution. The second topic will be considered briefly here.

Handwritten calculations and notes at the bottom of the page, including a large number 5625 and various smaller numbers and symbols.

GENETIC DRIFT

Genetic drift may be defined as the drift toward (or the selection of) a genetic type in numbers out of proportion to those found in the original stock. It is a consequence of sampling error. This does not mean that a "mistake" was made in choosing a small number of individuals from the original population to be the progenitors of the larger population. It merely means that it is impossible to select a very small sample of a population that will be representative of the whole population.

Suppose we had a comparatively small population of 16 individuals consisting of four A/A , eight A/a , and four a/a . The frequency of $A(p)$ is $\frac{1}{2}$ and that of $a(q)$ is $\frac{1}{2}$. Assume further that there were eight males and eight females, or eight pairs. If each pair left four offspring, the result would be a new population double the size of the first, with the gene frequencies remaining the same as the original, where $p = q = \frac{1}{2}$.

Instead of allowing all 16 individuals to mate, an individual pair might be picked out to become the progenitors of the new population. Suppose further that the phenotypes of the different genotypes were indistinguishable. The pair selected could have the different genotypes, with the gene frequencies shown in Table 20-4.

Table 20-4. All Possible Types of Matings in an F_2 Population, Segregating for Genes A and a

Genotype	Frequencies	
	p	q
$A/A \times A/A$	1.0	0
$A/A \times A/a$	0.75	0.25
$A/A \times a/a$	0.5	0.5
$A/a \times A/a$	0.5	0.5
$A/a \times a/a$	0.25	0.75
$a/a \times a/a$	0	1.0

Thus the gene frequencies of $p = q = \frac{1}{2}$ can be completely changed in one generation, if small nonrandom samples are selected to be the progenitors of the new population. Genetic drift, then, is the result of sampling error, plus the expansion of a small population into a large population.

A good illustration of genetic drift is the MN blood group series in man. We have seen that in one population the frequency of N genes (q) in the population was .75, while in another it was .24, almost completely the reverse. These differences could have arisen as a result of a sampling error in the original population, if we assume some standard base at the beginning. Other factors must be considered, such as a differential mutation rate or natural selection. It seems likely that choosing different samples of a small population to produce a larger one is the most likely answer.

POPULATION GENETICS EXPERIMENT
WITH DROSOPHILA

Instead of speculating on how different gene frequencies arise in man, let us consider another genetic tool, *Drosophila*, which has contributed so markedly to our understanding of genetics. *Drosophila* has a short generation time (10-12 days) and produces much larger populations than man, making it a better subject for a study of population genetics.

If we cross a scarlet-eyed *Drosophila* (*st/st*) by wild (+/+), the F_1 all will be +/*st*, having dark red eyes with the black spot characteristic of wild, because wild is dominant. In the F_1 , the frequency of + is .5 (*p*) and the *st* allele has the same frequency, .5 (*q*). The Hardy-Weinberg law tells us that, after one generation of random mating, the frequencies of *p* and *q* will remain the same, and they will be in the following distribution:

$$p^2 +/+ + 2pq +/st + q^2 st/st$$

Suppose a pair of F_1 flies has 400 offspring. The distribution expected is shown in Table 20-5.

Table 20-5. Distribution of Genes in F_2 Population where $p = q = 0.5$

Genotype	No. Individuals	+ genes	<i>st</i> genes	Total
+/+	100	200	0	200
+/ <i>st</i>	200	200	200	400
<i>st/st</i>	100	0	200	200
Total		400	400	800

If we should transfer all of the F_2 flies to a larger culture bottle and secure an F_3 generation, we would expect this relationship to continue.

If, instead of putting all the F_2 flies into one bottle, we take small samples of the F_2 to form many new populations, we will get populations in which the frequencies of *p* and *q* will vary greatly. We have seen three different genotypes in the F_2 . From these there are nine possible matings, which will occur in the frequencies shown in Table 20-6. Each of three different kinds of females can mate with each of three different kinds of males. The frequencies of the different kinds of matings will be the product of the frequency of the different genotypes in the F_2 , which is one fourth +/+, one half +/*st*, and one fourth *st/st* (Table 20-6).

It is evident that some of these matings are alike if we disregard the fact that some of the crosses are reciprocals which we know are alike, since the gene studied is autosomal (chromosome 3) and not sex-linked. There are but six totally different kinds of matings, since reciprocal crosses are alike (Table 20-7).

Table 20-6. Frequency of Different Genotypes and Possibilities for Random Mating of All Possible Genotypes

	Matings ♀ ♂		Frequencies of Genotypes		Frequency of Mating
(1)	$+/+ \times +/+$	$\frac{1}{4} \times \frac{1}{4}$	$\frac{1}{16}$
(2)	$+/+ \times +/st$	$\frac{1}{4} \times \frac{1}{2}$	$\frac{1}{8}$
(3)	$+/+ \times st/st$	$\frac{1}{4} \times \frac{1}{4}$	$\frac{1}{16}$
(4)	$+/st \times +/+$	$\frac{1}{2} \times \frac{1}{4}$	$\frac{1}{8}$
(5)	$+/st \times +/st$	$\frac{1}{2} \times \frac{1}{2}$	$\frac{1}{4}$
(6)	$+/st \times st/st$	$\frac{1}{2} \times \frac{1}{4}$	$\frac{1}{8}$
(7)	$st/st \times +/+$	$\frac{1}{4} \times \frac{1}{4}$	$\frac{1}{16}$
(8)	$st/st \times +/st$	$\frac{1}{4} \times \frac{1}{2}$	$\frac{1}{8}$
(9)	$st/st \times st/st$	$\frac{1}{4} \times \frac{1}{4}$	$\frac{1}{16}$

Let us now examine the frequency of genes $+$ and st to be expected from the six different kinds of matings, as shown in the last two columns of Table 20-7. In approximately one sixteenth of the cases there would be no st genes present. The whole population would consist of $+$ alleles ($p = 1.0$ $q = 0$). Likewise, in one sixteenth of the F_2 matings (No. 9), there would be no $+$ alleles ($p = 0$ $q = 1.0$). The population would breed true for scarlet.

Table 20-7. Summary of Frequencies of Possible Types of F_2 Matings, with Frequencies of Genes $+$ and st in this and Future Populations

Matings	Genotypes	Proportion	Gene Frequency	
			$p = (+)$	$q = (st)$
(1)	$+/+ \times +/+$ $\frac{1}{16}$	1.0	0
(2, 4)	$+/+ \times +/st$ $\frac{1}{4}$	0.75	0.25
(3, 7)	$+/+ \times st/st$ $\frac{1}{8}$	0.5	0.5
(5)	$+/st \times +/st$ $\frac{1}{4}$	0.5	0.5
(6, 8)	$+/st \times st/st$ $\frac{1}{4}$	0.25	0.75
(9)	$st/st \times st/st$ $\frac{1}{16}$	0	1.0
Total		1		

In approximately one fourth of the matings, Nos. 2 and 4, there would be three $+$ alleles and one st allele. The q value for st is .25. Likewise, in approximately one fourth of the matings, Nos. 6 and 8, there would be three st alleles to one $+$ allele. The value for q is .75. If large populations were developed from these matings, the q values of .25 and .75 would tend to remain constant. Their destinies were determined by taking the smallest sample possible (one pair of flies) from the original F_2 population.

The remaining three eighths of the F_2 matings (Nos. 3, 7, and 5) would result in populations in which the values of p and of q would be .5, the same as the F_1 and the same as the whole F_2 population.

Here, then, is a working sample of genetic drift. The later populations resulting from the isolation of individual pairs in the F_2 are different because of the lack of random sampling in the F_2 . Genetic drift is a consequence of sampling error in a small population, plus the expansion of a small population into a larger one.

The teacher of genetics could use the foregoing experiment in his class in genetics. If individual pairs of the F_2 flies (virgin female, of course) are given to the student at the beginning of the term, he could then allow these to produce the F_3 , and so on. During the semester the student should be able to raise eight or ten generations of flies. At the end of the semester he could analyze his population for p , the frequency of the wild (+) allele, and for the frequency of the *st* allele (q). No selection for eye color should be made during the course of the experiment. It is almost certain that different students will find different frequencies of the genes + and *st* at the end of the experiment.

POPULATION GENETICS OF SEX-LINKED CHARACTERS

Sex-linked characters show gene frequencies different from autosomal characters because the genes are located in the X chromosome. Since

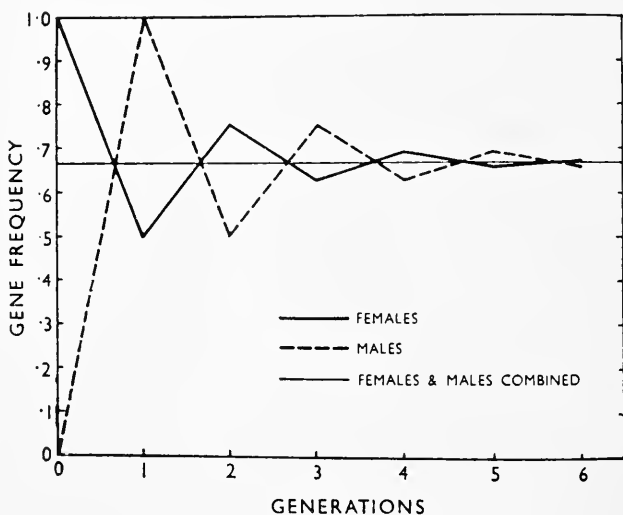
Table 20-8. Gene Frequency of Bar, a Sex-linked Gene

		No. of B Alleles	AA A+ a A+ A+ A+ A+
P....	$\left\{ \begin{array}{l} B/B \text{ } \text{♀} \dots\dots\dots 2 \\ \times \\ +/Y \text{ } \text{♂} \dots\dots\dots 0 \end{array} \right.$		
F ₁ $B/+ \text{ } \text{♀} \quad B/Y \text{ } \text{♂}$			
No. B alleles in F ₁ ♂ and ♀		2	
Total X chromosomes in F ₁ ♂ and ♀		3	
Frequency of B allele.....		$\frac{2}{3}$	
Reciprocal hybrid			
		No. B Alleles	
P....	$\left\{ \begin{array}{l} +/+ \text{ } \text{♀} \dots\dots\dots 0 \\ \times \\ B/Y \text{ } \dots\dots\dots 1 \end{array} \right.$		
F ₁ $B/+ \text{ } \text{♀} \quad +/Y \text{ } \text{♂}$			
Total B alleles in F ₁ ♀ and ♂		1	
Total X chromosomes in F ₁ ♀ and ♂		3	
Frequency of B allele in F ₁		$\frac{1}{3}$	

females have two X chromosomes and males but one, it is evident there can never be a gene frequency of one half, as can exist for autosomal traits. The gene frequency can only be some one of the following: one, two thirds, one third, or none.

For example, if a mating is made between a *Drosophila* female homozygous for Bar eye and a wild male, the gene frequency of the Bar allele in the F_1 will be two thirds. This can be seen in the upper part of Table 20-8 on page 379.

These frequencies of the Bar allele will remain constant in future generations when both males and females are considered. In this case the frequency of the Bar allele will depend upon which way the original cross was made. This applies for any sex-linked trait. When introduced into the population



Courtesy of Falconer; and Oliver & Boyd

FIG. 20-2 Approach to equilibrium under random mating for a sex-linked gene.

through the homozygous female, the frequency of the sex-linked gene will be two thirds, or .67. If introduced into the population through the male (with only one X chromosome) the gene frequency will be one third (.33).

These frequencies exist for the parent, F_1 , and all subsequent generations when both males and females are considered. Strikingly different gene frequencies exist for a few generations in males and females.

This is well illustrated by a graph in *Quantitative Genetics*, by D. S. Falconer (Fig. 20-2). Note the zig-zag appearance of the lines for females and males in the F_1 and succeeding generations, while the combined frequencies of the mutant allele remains at .67. Had the mutant been introduced by the male, the frequency would have been .33.

CHANGE IN GENE FREQUENCIES BECAUSE OF RECESSIVE LETHALS

A common type of mutant gene in almost any population is a recessive that is lethal when homozygous. What effect on a population would there be through the elimination of the recessive lethals? We might ask the question, what decrease in the gene frequency would there be for any recessive gene (not lethal) if the homozygous recessive individual were kept from reproducing? The gene for albinism is a good example. If all men and women who are albinos were sterilized, how much would this reduce the frequencies of albinos? This type of program was once advocated as a good method of eugenics, a popular mode of improving the human race before the advent of population genetics. We will return to the sterilization proposal after examination of some population genetics with corn, involving a gene for albinism, or white seedlings. The albino in corn is lethal, possessing no chlorophyll; hence it is self-eliminating.

POPULATION GENETICS OF WHITE SEEDLINGS IN CORN

There are many different genes causing white seedlings in corn. If a plant heterozygous (+/w) for the white seedling is self-pollinated, the resulting seed will produce seedlings in the ratio of three green to one white. The genotype of these plants is as follows:

$$1 \text{ } ++ : 2 \text{ } +w : 1 \text{ } ww$$

The white seedlings die, so the green seedlings represent the viable population in the proportion of $\frac{1}{3} ++ : \frac{2}{3} +w$. Since one half of the alleles in the +/w plants are w, the frequency (q) of w is $\frac{1}{2} \times \frac{2}{3} = \frac{1}{3}$. The frequency of + is $1 - q = \frac{2}{3}$. If all of the green plants (the only kind surviving) are allowed to interpollinate and the ears from this field shelled *en masse*, it is easy to calculate by the Hardy-Weinberg Law the proportion of the different genotypes in the next generation. This is given by the formula:

$$p^2 ++ + 2pq +w + q^2 ww =$$

$$\left(\frac{2}{3}\right)^2 ++ + 2\left(\frac{2}{3} \times \frac{1}{3}\right) +w + \left(\frac{1}{3}\right)^2 ww,$$

which results in the following proportions,

$$\frac{4}{9} ++ + \frac{4}{9} +w + \frac{1}{9} ww$$

Thus it is seen that one ninth of the seedlings are lethal and that the viable population consists of equal parts of ++ and +/w plants, or in a ratio of one half to one half. Since one half of the alleles in the +/w plants are w, and the q for w in the viable population is one half of one half, or one fourth.

In three generations of random mating, the frequency of genes for white seedlings has decreased from one half, to one third, to one fourth. It can be demonstrated that in the next generation the value is one fifth, then one sixth, and so on indefinitely.

Li (1955) has devised a formula for this progressive decrease in the frequency of a recessive lethal gene. It is as follows:

$$q_{n+1} = \frac{q_n}{1 + q_n}$$

Substituting the figures in the first example where $q = \frac{1}{2}$, we get:

$$q_{n+1} = \frac{\frac{1}{2}}{1 + \frac{1}{2}} = \frac{\frac{1}{2}}{1\frac{1}{2} \text{ or } \frac{3}{2}} = \frac{1}{2} \times \frac{2}{3} = \frac{1}{3}$$

When the gene frequency of the recessive is comparatively high, one half in this instance, the change in the succeeding generation is fairly great, from one half to one third. If, however, the frequency is rather low at the start, let us say $\frac{1}{100}$, one generation decreases the frequency to only $\frac{1}{101}$. The rate of decrease is shown graphically for the first 60 generations in Fig. 20-3.

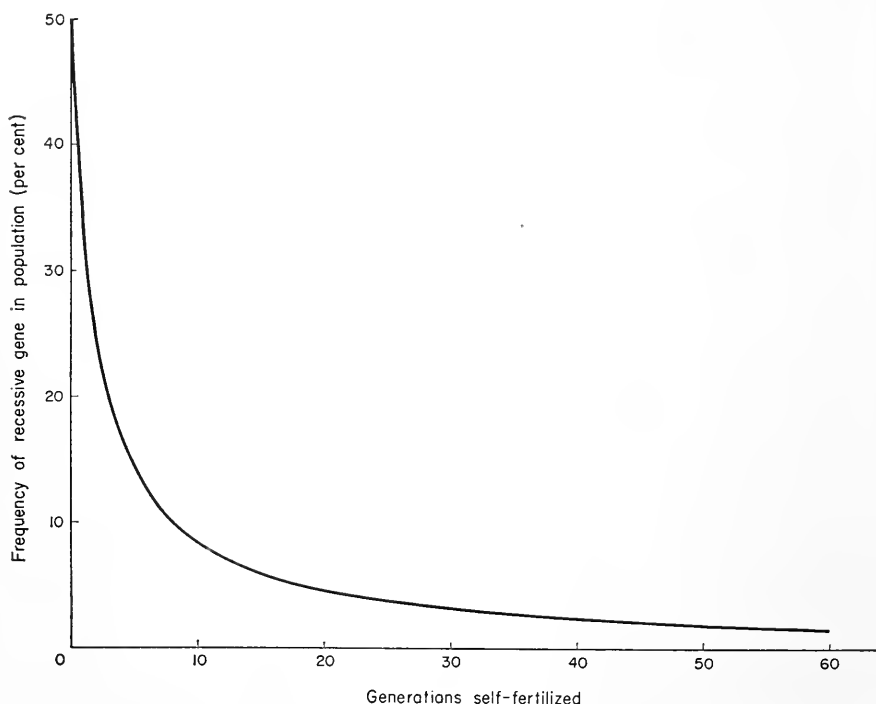


FIG. 20-3 Decrease in the frequency of a recessive gene in the population when double recessive zygote is lethal, represented by the formula $q_{n+1} = \frac{q_n}{1 + q_n}$.

Advocates of eugenic measures to eliminate a harmful recessive gene from a human population by sterilizing the recessive individuals might be encouraged if they considered only the first part of this graph, say the first ten generations.

However, when the initial gene frequency is low, a great many generations are required to reduce it by half, and it is never eliminated from the population. Take the gene for albinism in man, as was mentioned. If we assume the frequency of $\frac{1}{100}$, 100 generations would be necessary to reduce it to $\frac{1}{200}$. If we assume a generation time in man to be 30 years, the period would be 3000 years, not to eliminate albinism, but merely to reduce its frequency one half. In other words, if all of the albinos in a population had been sterilized since 1000 B.C., the frequency of this gene in the population would be only one half as great as now. This does not take into consideration new mutations to albinism which would at some point equal the number eliminated through sterilization.

Thus a knowledge of population genetics shows how futile is a program once advocated for race improvement.

GENETIC DESTRUCTION OF AN ENTIRE POPULATION, THE CASE OF THE SCREW WORM FLY

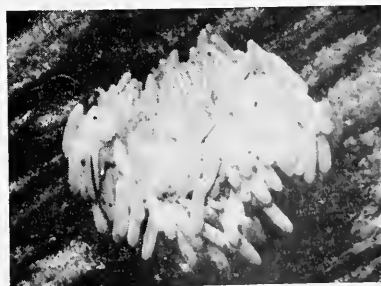
Population genetics usually is concerned with gene frequencies in a population, and the conditions that may cause a certain gene to increase or decrease. The population itself remains an entity, despite the fluctuations of the various genes within.

The experiment to be described below is concerned with the complete elimination of a population by genetic means. It is often referred to as the sterile-male method of population control. This is the interesting work of an entomologist, E. F. Knipling, and some of his colleagues in the United States Department of Agriculture. They employed the clever technique of irradiating male insects sufficiently to sterilize them, so that when mated with normal females no viable offspring resulted. The insect on which this was used with outstanding success was the screw worm fly, *Callitroga hominivorax* (Fig. 20-4). It lays eggs on the backs of farm animals (Fig. 20-5). The larvae develop be-



Courtesy of E. F. Knipling; and the
United States Department of Agriculture

FIG. 20-4 Adult screw worm flies, male (left) and female (right). The screw worm is about three times as large as the common house fly.



Courtesy of E. F. Knipling; and the
United States Department of Agriculture

FIG. 20-5 Egg masses of screw worm fly. Females deposit 200 to 300 eggs on the edges of wounds in the host cattle.

neath the skin of sheep, goats, and cattle, causing them to lose weight or to gain very slowly in comparison with noninfested animals. Also, the hides of the infested animals are seriously damaged.

In 1950 this unique experiment in insect control was initiated, following consultation with Muller. Bushland and Hopkins (1951) determined that male screw worm flies could be sterilized with 2500 r from X rays. Sterility in the female required 5000 r.

Males sterilized by this method mated with untreated females, but produced no offspring. The mating habits of such males were essentially the same as for normal males. In competition with normal males in ratios of one to one and nine to one for sterile to fertile males, the ratio of sterile to fertile egg masses was approximately the same as the ratio for males used.

This was demonstrated by mixing sterile males with normal ones in different proportions and observing the proportion of female flies that did or did not produce egg masses. When the ratio of sterile to fertile males was either one to one or nine to one, the ratio of female flies producing no egg masses or producing egg masses was approximately the same as the ratio of sterile to fertile males.

Proof of the experiment was to liberate a large number of sterilized flies in the breeding area. How this method operates is shown in Table 20-9 (courtesy of Knippling).

Table 20-9. Theoretical Effect of Release of Sterile Individuals on a Screw Worm Fly Population

Generation	Number Normal Insects in Population	Number Sterile Insects Released	Ratio Sterile to Fertile Insects
P	1,000,000	2,000,000	2 : 1
F ₁	333,333	2,000,000	6 : 1
F ₂	47,619	2,000,000	42 : 1
F ₃	1,107	2,000,000	1807 : 1
F ₄	<1	2,000,000	> 2,000,000 : 1

By making repeated releases of 2,000,000 sterilized insects (both males and females) over an entire breeding season, it was possible to eliminate completely the population of screw worms in the small island of Curaçao in the West Indies. Within seven weeks after starting the experiment, the percentage of sterile egg masses recovered from the samplings had risen to 100. No fertile masses were found.

The method used in Curaçao has been applied successfully to Florida. It has destroyed completely the screw worm in this state, and saved livestock growers there millions of dollars annually.

Thus a unique combination of radiation genetics and population genetics completely eradicated an insect pest from its native habitat.

CONCLUDING REMARKS

Chapter 20 is concerned with the frequencies of genes in a population. The Hardy-Weinberg law states that after one generation of random mating the population is in equilibrium and the relative frequencies of the genes will remain constant. Methods of calculating gene frequencies were presented.

The change in the frequency of lethal genes in a population was discussed. These are reduced very slowly, and never eliminated.

Genetic drift is change in a population because of nonrandom sampling and the subsequent increasing of such nonrandom samples to form larger populations.

The interesting example of the complete elimination of the population of the screw worm fly, by genetic means, was presented.

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PROBLEMS

- 20-1. Define or describe the following terms:
- | | |
|---------------------|---------------------|
| gene frequency | random mating |
| genetic drift | screw worm fly |
| Hardy-Weinberg Law | sterile male method |
| non-random mating | (screw worm fly) |
| population genetics | |
- 20-2. Identify the following scientists, giving a major contribution with an approximate date.
- | | |
|-----------------|-----------------|
| Bushland, R. C. | Knipping, E. F. |
| Falconer, D. S. | Li, C. C. |
| Hardy, G. H. | Weinberg, W. |
| Hopkins, D. E. | Wright, Sewall |

- 20-3. In a genetics class there were 13 tasters and seven non-tasters. Non-taster is recessive t/t . What is the frequency of the t gene in this small population? (You may shift the percentage of t/t persons one per cent either up or down to make calculation easier.)

How many of the $T/-$ persons would be expected to be T/T , and how many T/t . Express to the nearest whole number.

- 20-4. In a hypothetical population 16 per cent are non-tasters. If two tasters marry, what would be the probability of their having 5 children all of whom were non-tasters? If the 5 children were quintuplets like the identical Dionne quintuplets (born in 1934) what would be the probability?

- 20-5. In people classified for the MN blood groups it is possible to calculate directly the frequency of both M and N alleles in the population. Calculate the gene frequency for the M and N alleles in the following population:

119 M 76 MN 13 N

If the population is in equilibrium the frequency of the genes in the population can be described by the formula

$$p^2 M + 2 pq MN + q^2 N$$

Demonstrate that the population either is or is not in equilibrium.

- 20-6. Assume that in a population of 1000 individuals the following blood types were found: 400 M , 400 MN , and 200 N .

What is the frequency of gene M , of gene N ?

Is the population in equilibrium?

What is the expected distribution of the three genotypes with a population in equilibrium?

Calculate the chi-square for the observed distribution and tell whether you think this verifies or denies the assumption that the population is in equilibrium.

What is the probability that the population is in equilibrium? See Table 5-2 (in Chapter 5).

- 20-7. An investigator self-pollinated an ear of corn heterozygous for white seedlings ($+/w$) and planted the seeds in a small plot isolated from all other corn. There were 300 green seedlings and 100 white, which lived but a short time. What was the frequency (q) of the w allele in the selfed ear?

- 20-8. What is the frequency (q) of w in the population of 300 remaining green plants (Problem 20-7) after the white seedlings have died?

- 20-9. The plants in this field (Problem 8) were allowed to interpollinate, and seed from all plants was saved. A population of 10,000 green plants (plus a considerable proportion of white seedlings) resulted the following year.

How many white seedlings were there?

What is the frequency of the w allele in these 10,000 green plants?

- 20-10. How has the frequency changed from (a) the original ear, to (b) the small plot of 300 plants, and to (c) the larger plot of 10,000 green plants? What is the formula for this decrease in the frequency of w ?

- 20-11. If bulk seed were saved from the last field, what proportion of the seeds would produce white seedlings?

- 20-12. In the preceding problems the gene for white seedlings was selected against, since no w/w plant ever lived to maturity. Yet the w allele persisted in the population. If the frequency of w were $\frac{1}{40}$, how many years would it require to reduce it to $\frac{1}{20}$ (assuming a plot of corn were grown each year)? How many years would it require to reduce the frequency from $\frac{1}{20}$ to $\frac{1}{40}$?

- 20-13. In *Drosophila* the genes for cinnabar eye color (*cn*) and vestigial wings (*vg*) are located approximately 10 units apart in chromosome 2. Diagram a cross between these two genetic types, writing the genotypes of both parents and the F_1 and giving the proportions of all the segregates in the F_2 . Utilizing your knowledge of population genetics, devise a method for producing a homozygous double recessive *cn vg/cn vg* and tell in what frequency this type will be obtained. Remember there is no crossing-over in the male.
- 20-14. If the frequency of the gene for albinism (*c*) in a human population were .01, how many generations would it take to reduce it by one-half to .005 if all the albinos in the population were sterilized? Assuming a generation time in man to be 30 years, how many years would it take to reduce the frequency of *c* by one-half (from .01 to .005), not considering the mutation from *C* to *c*, which might be considerable?
- 20-15. The dilution gene *D* producing a palomino horse when in the heterozygous condition *D/d* is an excellent one for studying population genetics, since there is no dominance and all three genotypes *D/D*, *D/d* and *d/d* have characteristic phenotypes. (See Chapter 10.) Suppose a horse breeder bred 50 chestnut mares (*d/d*) to a cremello stallion (*D/D*) and produced 50 palomino foals (*D/d*), with an equal number of male and females. If these foals, when grown, were all placed on an isolated island and allowed to inter-breed, what would be the values of *p* and *q*, after a period of 50 years?
- 20-16. Instead of having all 50 palominos on the island there were 40 palominos and 10 chestnut horses, again with an equal division of the sexes. After 50 years what would be the values of *p* and *q*?
- 20-17. Make a similar calculation for 10 palominos and 40 chestnut horses.

Genetics and Evolution

IN THIS CHAPTER we are concerned neither with the fact that evolution exists nor in presenting data showing that it exists. Rather our interest lies in how the science of genetics has contributed to our understanding of the way in which evolution has come about. Genetics has given us an understanding of the method whereby evolution could take place.

The basic building block of evolution is mutation. But mutation is not enough—there must be ways of getting the mutant gene into populations. This is the role of natural selection.

This theory was proposed more than a century ago by two scientists independently of each other. Charles Darwin and Alfred Wallace separately arrived at the conclusion that natural selection is the force sorting out the variations and perpetuating them in varieties, subspecies, and species. Their studies came before Mendel's experiments, and they had no precise knowledge about the way variations took place. Darwin held some ideas, now discredited, as to how hereditary traits were passed on. His theory of pangenesis assumed that there were "gemmules" in the body which in some way governed hereditary traits passed on from generation to generation. However, even with little precise knowledge of the mechanism of heredity, the natural selection theory of Darwin and Wallace provided an explanation for evolution.

Darwin expanded his views in the *Origin of Species by Natural Selection*, first published in 1859. An excellent appraisal of this work was made by Muller in a talk entitled "Genetics and Evolution." This is stated with clarity and conciseness. We publish a few extracts:

. . . Charles Darwin's work, *The Origin of Species by Natural Selection*, published one hundred years ago can justly be considered as the greatest book ever written by one person. The penetration of its analyses in previously unworked and recondite fields is matched by the range and comprehensiveness

of its treatments of all then known aspects of living nature and by the masterliness of its integration of these diverse elements into its coherent, unifying scheme. With this one grand sweep Darwin laid bare the basic principles by which all past and present day organisms, including man, have been gradually generated from the simplest beginnings of animate material, in the course of development that he termed the Tree of Life. He thereby showed the branching form of their interrelations with another and—most important of all—the manner in which the superlative complexities of their adaptations for survival and reproduction have arisen by the operation of purely natural causes, of a kind still working today.

For men's understanding of the nature of life in all of its multitudinous expressions, all this truly amounted to a revelation. It gave for the first time a solid scientific basis in natural processes for these phenomena, including the nature of man himself: phenomena which had seemed to be utterly teleological, that is, directly contrived for the purposes that they seemed to subserve. In bringing about an understanding of this matter, Darwin's book effected a greater revolution in men's conceptions of the universe and of themselves than any that had come before it or, indeed, than any that have as yet come afterward. What volume has been so creative as this?

Perhaps because Darwin sensed the opposition that such an innovation in outlook would be certain to arouse, he concealed the profound significance of his work under an innocent sounding title that seems to confine its subject matter to the level of mere species differences. This title, *The Origin of Species*, is surely one of the greatest masterpieces of British understatement. Its modesty is further understandable in the light of Darwin's curious inferiority complex. He well realized, however, that, once the species barrier is surmounted no obstacle nearly as serious remains to hinder an unlimited evolutionary movement, a movement that can eventually take life forms all the way along the great courses of their phylogenetic history. And in the book itself he brought about these consequences clearly.

Now the great key to this whole picture is the principle of natural selection, or survival of the fittest. On casual consideration this appears to us in our day as almost a truism. It is true that before the expositions of it by Darwin and by Wallace it had been glimpsed on rare occasions. However, these previews of it were fragmentary in their nature and limited in their application. It was in the universalization of it into an interpretation of living things in general, and in the marshalling of an enormous amount of evidence for it, that the greatness of Darwin's and, to a lesser extent, of Wallace's contributions lay.

We are not primarily concerned in this discussion with the diverse kinds of evidence for evolution gathered together by Darwin: through comparisons of present day living things with one another in regard to details of their morphology, physiology, ecology, and embryology, nor through studies of their geological succession, nor even through the analogies he drew with the effects of domestication and cultivation as practiced by man. All these studies were, to be sure, enough to make any other than an evolutionary interpretation entirely unreasonable. Neither are we primarily concerned here with the enormous extensions of such lines of inquiry that have been carried out since Darwin's time, nor with the unequivocal substantiation of this interpretation that they have provided. We shall take this all for granted. What does concern us directly here is, firstly, the entry into the picture of the methods and findings of genetics, in confirming and elucidating Darwin's and Wallace's key discovery of natural selection, and, secondly, the manner in which genetics, carrying these analyses further, has pointed the way to a liaison of biology with the

chemistry, physics, and mathematics "beneath" it and with the social sciences "above" it.

THE GENETIC NATURE OF NATURAL SELECTION

Natural selection is the most important process in the evolution of new types in a population. It is sometimes called the "survival of the fittest" and has as its basis the following premises:

1. All species of plants and animals produce more progeny than can possibly survive in any given habitat.
2. There is variation in the progeny.
3. Only a portion of the progeny can possibly survive *to leave progeny*.

The last criterion is the crux of the problem. The test of an animal's fitness is not how big or strong he is, but how many progeny he leaves. From an evolutionary standpoint a lion's speed is essential, not so much for insuring that the lion himself escapes harm and is able to capture food, but because it insures the begetting of progeny. All the speed and stamina in the world are of no avail if the genes of the bearer are not passed on.

To illustrate this point, we could well consider the mule, which is a hybrid of the horse and the ass. It has been said that a mule has more horse sense than a horse and more stamina than either parent. As an animal for hard work he is fit. Yet from an evolutionary standpoint he represents a dead end. He is sterile and can not pass on any of his genes to future populations. In this sense he is not fit at all, in contrast to his parents who can and do leave progeny.

EVOLUTION—ONE PHASE OF POPULATION GENETICS

This chapter is really a continuation of Chapter 20, "Population Genetics." It was only after the establishment of population genetics, following the two outstanding papers of Hardy and Weinberg, that the science of genetics was able to explain how evolution could have come about.

One of the tenets of the Hardy-Weinberg Law is that populations tend to remain at equilibrium in regard to frequencies of different genes. The frequency of genes in the population remains constant from generation to generation regardless of the relative frequency of two alleles in the population. This will be discussed more fully in the next section.

GENETIC EQUILIBRIUM IN POPULATIONS

One type of genetic equilibrium in a population is that mentioned in Chapter 20. This concerns the constancy of the initial gene frequencies from one generation to another generation. These frequencies, p and q , may be equal, in which case $p = q = .5$ since $p + q = 1$. In a mating of homozygous

The frequencies of p and q may differ greatly. In one population segregating for the MN series of blood groups noted in Chapter 14, the frequency of gene M was .76, while that of gene N was only .24. These frequencies would tend to remain the same under random mating.

As we said before, mutation is the basic building block for evolution. It provides the raw material. That all genes mutate is axiomatic. The mutation rates may vary and they do. Not only that, but reverse mutations occur. As A mutates to a , so a mutates back to A , usually at a lower rate. Let us assign these mutation rates a mathematical value and develop a model for explaining gene frequencies that have different values.

$$A \xrightarrow{f} a$$

$$A \xleftarrow{b} a$$

The frequency of gene A in the next generation will be as follows:

The population will be at equilibrium when the additions just balance the subtractions.

$$bq = f(1 - q)$$

A little algebraic transposition shows us that this equals:

$$bq = f - fq$$

$$bq + fq = f$$

$$q(b + f) = f$$

$$q = \frac{f}{b + f}$$

We have set down this algebra so that we may assign values to b and f and consequently obtain a value for q , the frequency of the mutant allele a in the population when genetic equilibrium has been reached. Now let us suppose that the rate f is three times that of rate b , in other words, $f = 3b$. Substituting these values in the formula, we get:

$$q = \frac{3b}{b + 3b} = \frac{3b}{4b} = \frac{3}{4}, \text{ or } .75$$

Since $q = .75$, $p = .25$.

With these rates of mutation the population will reach equilibrium when the frequency of gene A is .25 and that of a .75. These values represent the proportion of the two alleles under mutation pressure, in both directions.

This is a fundamental kind of equilibrium. It takes into consideration the mutation pressures, forward and reverse, in establishing the gene frequencies of a population. It is evident that this is a basic equilibrium of importance in understanding the effect of mutation pressure.

NON-RANDOM MATING

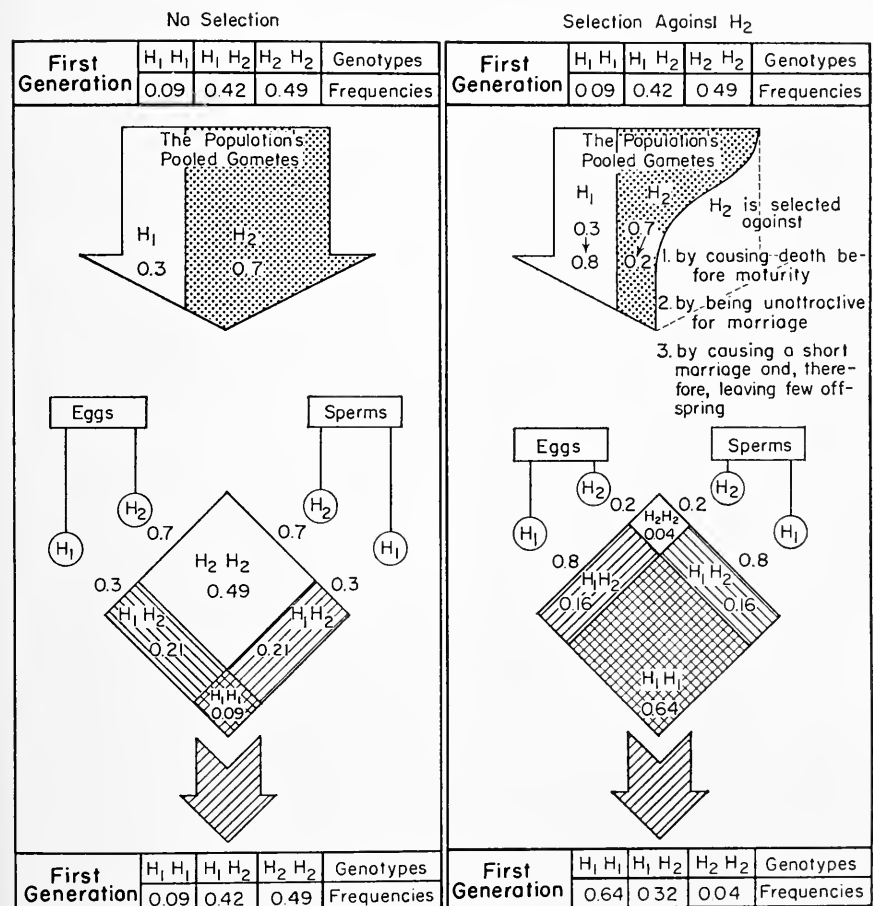
In the foregoing examples we have assumed that the mating was at random, and that the mutant gene a is fully as viable as A , leaving proportionately as many progeny. This is not always true. You remember the example of the gene for white seedlings in maize in Chapter 20. This gene is lethal when homozygous and the homozygous recessives (w/w) leave no progeny. We saw the effect of this condition on the progeny left by a population, beginning with the selfed progeny of a single heterozygous $+/w$ plant. The frequency of w (q) decreased from $\frac{1}{2}$ to $\frac{1}{3}$ to $\frac{1}{4}$, etc., in succeeding generations from mass pollinations of the whole population. The formula for this decrease, as pointed out in Chapter 20, is

$$q_{n+1} = \frac{q_n}{1 + q_n}$$

EFFECT OF NON-RANDOM MATING ON INCIDENCE OF HEMOPHILIA

Let us now consider an example in which the mutant gene is at a serious disadvantage, but the homozygote is not lethal as in white seedlings

of maize. This is the sex-linked inheritance of hemophilia in man. Males with one gene for hemophilia (*h*) are affected, since the gene is located in the non-homologous part of the X chromosome and males are hemizygous. Females must be homozygous (*h/h*) to have the disease.



Courtesy of Simpson, Pittendrigh, and Tiffany; and Harcourt Brace

Fig. 21-1 Effect of natural selection on a subvital gene in a population. H₂ is the gene for hemophilia. The chart represents females only. Males would show the same ratio as gametes, since males are hemizygous.

Although the gene is not lethal, it does cause a departure from random mating. Males with hemophilia seldom survive to the reproductive age. If they do mature, they rarely marry and leave as many progeny as normal males. The same would be true of homozygous females *h/h*, who, however, are extremely rare. Both parents of such an *h/h* person must have had at least one *h* allele. Or possibly the mother was heterozygous *H/h* and a mutation from *H* → *h*

might have occurred in the sperm of the father. It is known that the mutation rate from $H \rightarrow h$ is rather high, about 2 or 3×10^{-5} , about 1 in 33,000 to 50,000. The backward mutation rate from $h \rightarrow H$ is not known, but it is certainly much lower than the forward rate.

With a comparatively high forward mutation rate f and a low backward rate b , the hemophilia condition should be rather common in man. Actually, the incidence is only about one in 10,000, much less than would be expected with a high mutation rate in operation over a long period of time.

The reason for the discrepancy is the deleterious effect of the h allele on males who have it. The selection against the h gene is illustrated for females in a population in Fig. 21-1. The checkerboard is used here to show all the genes in the population, rather than to illustrate the possible gametes of an individual genotype. Such a population genetical checkerboard is limited to females, in the case of hemophilia, since it is a sex-linked condition. The gene frequency for males would be the same as for the gametes.

SICKLE CELL TRAIT—SELECTIVE VALUE

A gene that possesses some selective advantage in the heterozygous condition is the one causing a sickle-shaped hemoglobin cell, instead of the normal disk-shape one. (This was discussed in Chapter 19.) Homozygous normal individuals are h^a/h^a , heterozygote persons h^a/h^s (sickle cell trait), and homozygous individuals with sickle-shaped hemoglobin h^s/h^s (sickle cell anemia). This condition is found almost exclusively in the Negro race. The persons with sickle cell anemia h^s/h^s require frequent transfusions and rarely live to the reproductive age.

This is somewhat analogous to the gene for white seedlings in maize, which prevents the recessive homozygote from reproducing. Consequently one might expect a gradual reduction in the frequency of the h^s gene in the population. However, the frequency in certain populations is rather high. It has been found that heterozygous individuals h^a/h^s have greater resistance to malaria than homozygous normal persons h^a/h^a . Apparently this attribute of individuals with the sickle cell trait h^a/h^s has kept the h^s gene in the population in greater frequency than would occur otherwise.

Just why the heterozygous individuals have more resistance to malaria is a question. One theory is that the sickle-shaped red blood cells do not carry as much oxygen as the normal ones and may not supply the malaria parasite, *Plasmodium falciparum*, with sufficient oxygen for maximum growth.

ELIMINATION OF WHITE EYE IN DROSOPHILA POPULATION EXPERIMENTS

An interesting experiment in selective mating of *Drosophila* compared the competitive mating between white-eyed flies and the wild type with

red eyes (Reed and Reed, 1950). The experiment began with four virgin flies of the following genotypes: W/w and w/w females and W/Y and w/Y males. The gene frequencies in this population can be calculated as shown in Table 21-1.

Table 21-1. Gene Frequencies of W and w in Population Experiments

Genotype	Genes	
	No. W	No. w
4 W/w ♀ ♀	4	4
4 w/w ♀ ♀	0	8
Genes in females	4	12
4 W/Y ♂ ♂	4	0
4 w/Y ♂ ♂	0	4
Genes in males	4	4
Total genes in population	8	16

Gene frequency of $w = \frac{16}{24} = \frac{2}{3}$ or .67 w .

The frequency of the w gene in the population was .67. In the first generation it was observed that the white males were selected against, especially by wild-type females. This is shown in Table 21-2 (Table I of Reed and Reed).

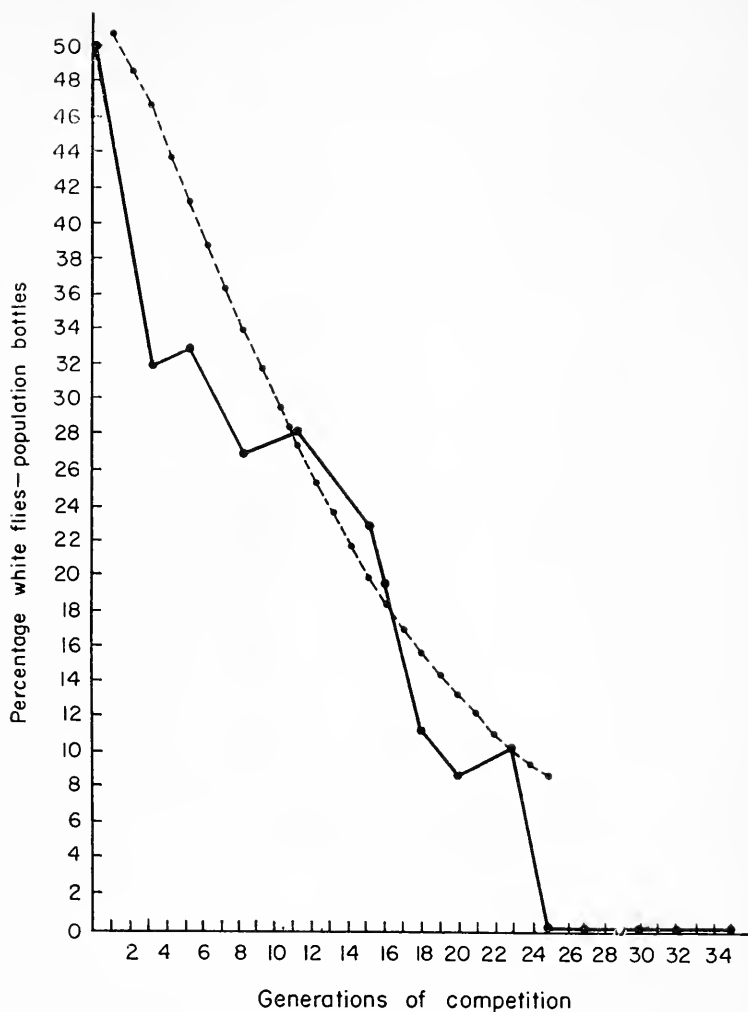
Table 21-2. Results of Selective Matings when Both Red and White Phenotypes of Both Males and Females are Present

	White ♀	Red ♀	Totals
White ♂	90	64	154
Red ♂	100	105	205
Double mating	6	4	10
No offspring	116	118	234
Total	312	291	603

Red males were almost twice as successful as white males in mating with red females. The preference of the white females was not so striking, although red males were preferred.

If the number of double matings is added to each of the two single ones, totals of 164 and 215 are obtained for a total of 379 matings. The ratio of wild-type males to white males is approximately 1.00 to .75. Using this proportion, an estimated extinction curve was calculated. It is shown in Fig. 21-2, in comparison with the observed rate of decrease in the number of white phenotypes in the population.

Actually, there were no white phenotypes in the progeny after 25 generations and none appeared in the next ten generations, so it was safely assumed that the gene w had been eliminated from the population by *competitive mating*. This is in contrast to the lethal gene for white seedlings in maize (w) which would never be eliminated from the population with random mating, even for a character that was lethal when homozygous w/w .



Courtesy of Reed and Reed in Evolution

FIG. 21-2 Competition to extinction in *Drosophila*. Wild-type males preferred in matings to white (w) by both wild (+) and white (w) females. Solid line observed curve, broken line calculated on ratio of 1.00 to .75 for wild and white males, respectively

COMPETITIVE SEXUAL SELECTION IN MICE

An interesting mouse experiment in preferential mating showed that albino females had a strong preference for albino males in competition with the wild-type agouti (Levine, 1958). An albino female was placed in a cage with two males, one an albino, the other agouti. The young were destroyed after they had been scored for color. Since the mice fathered by agouti

males have pigmented eyes they could easily be distinguished. Ten litters from ten different females, or 100 litters in all, were scored. The results are shown graphically in Fig. 21-3. In all cases there was a strong preference for the albino males.

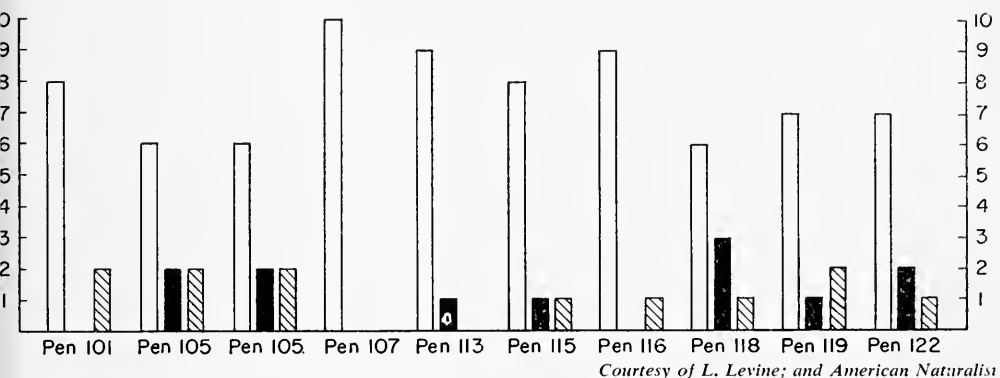


FIG. 21-3 Competitive selection in mice. Albino females showed preference for males of same phenotype. Open bars represent mating of two with same genotype, solid bars mating of different genotypes, and cross-hatched bars mixed matings. Female could choose between two males, one an albino, the other agouti.

This experiment and the previous one cited for *Drosophila* show natural selection in operation in laboratory populations. Neither of these non-random mating experiments could be possible under field conditions, but undoubtedly the factors that operated in the laboratory experiments would also apply to field populations.

It is interesting to reflect on the population of albino squirrels cited in Chapter 1. More than 700 are now in the colony, which arose as the progeny of a single pair of albino squirrels liberated a half century ago. There has been some "non-natural selection" involved in the evolution of this colony, since it is protected by law. The hand of man has had an effect here.

THE NATURE OF NEW MUTANTS

During the first half of the present century many studies of spontaneous and induced mutations have been made. Such studies reveal that most mutations are recessive in nature, and are somewhat deleterious either to the gamete or to the zygote when homozygous for two recessive alleles of the mutant gene (m/m). Not only that, but many recessive genes have a somewhat deleterious effect in the heterozygous condition M/m .

It is not surprising that the newly formed mutants are less fit than the genes already established. The plant or animal species has existed for millions of years. During its long history the species has incorporated the mutant genes

that contribute to its well-being and render it able to adapt to its given environment. During the long course of evolution, undoubtedly, countless mutations have arisen. Presumably the spectrum of such mutants is much like a sample of mutants studied over the last half century in the laboratory. The species has incorporated the "beneficial" genes and discarded the "detrimental" ones.

INTERACTION AMONG GENES HAVING SELECTIVE VALUE

We have presented a few cases of the behavior of mutant genes and how they may be altered by natural selection, which can be defined (to quote Muller): "the differential multiplication of variant (or mutant) types. And the genetic material is that core within living matter that enables it to accumulate, in virtually unlimited amount, these variations that undergo multiplication (that is, replication)" Muller, 1960.

The examples of mutant genes chosen to represent examples of non-random mating were more extreme in nature than the host of genes showing less striking phenotypic manifestations, but just as essential to the organism. There is every reason to believe that the principles shown for the white-eye gene in *Drosophila* and the albino gene in mice would be applicable to all genes.

We know that the genes for white eye and albinism do not act by themselves, but in cooperation with a great many genes that help to produce an individual in which the white-eyed phenotype or albino animal may be expressed. In Chapter 9 we discussed several cases of genic interaction in which the phenotypic effect was produced by the cooperation of different genes—complementary, duplicate, and triplicate genes. In Chapter 11 we discussed quantitative inheritance in which several cumulative genes contribute to a phenotypic effect. It is reasonable to suppose that genic interactions of these kinds also are present in genes contributing to the well-being of a species—those that have evolutionary significance.

GENE FLOW IN POPULATIONS

Any mutant gene, if it is to have evolutionary significance, must become incorporated in the population. It can become established by genetic drift and by natural selection. We might choose an example of a somewhat deleterious mutant, the one for albinism, and see how it becomes established in a population. Suppose we had a population of 50 wild rabbits, 25 ♀♀ and 25 ♂♂, and in one animal a single mutation from $C \rightarrow c$ arose. Its frequency in the population would be $\frac{1}{100}$, since there are 100 total genes at the C locus in the population. (Actually we should say 100 alleles of the C gene, but in all gene frequency analysis it is customary to speak of genes rather than alleles.)

Suppose this population of rabbits were isolated on an island and allowed

to interbreed and increase. For the purpose of the experiment, let us suppose that each rabbit pair produced four mature offspring, and in each succeeding generation the same ratio occurred. One of the 25 pairs would have one mutant allele, while the others would be C/C . The animal possessing the mutant would produce an equal number of C and c gametes. These must all find a C gamete to unite with, since there is only one heterozygous animal. In the new population of 100 there would be two c alleles in a total population of 200 genes, with a frequency of $\frac{2}{200}$ or $\frac{1}{100}$, which is the same as the beginning. It is possible now to have a mating that would produce an albino individual. The frequency of such an individual would be $\frac{1}{100} \times \frac{1}{100}$, or one in 10,000. This ratio would remain constant, subject to the back mutation and forward mutation discussed previously in this chapter. The example of the 50 rabbits is given in detail to illustrate how newly mutated genes find a phenotypic expression.

ISOLATING MECHANISMS

To be perpetuated, it is not necessary for newly arisen mutants to be isolated from the parental population. In the case of albino mutants in many species, the gene is carried from several to many generations in the heterozygous condition before it finds expression in an albino phenotype. Undoubtedly most genes respond in the same way.

However, if new species are to be formed, there must be different ways of isolating segments of a divergent population. An illustration of how an environmental change might establish a new mutant population is found in the mutant of the small water flea, *Daphnia*, which was adapted to a higher temperature (discussed in Chapter 6). Once the new types have been increased, how are they isolated into new populations, be they varieties, races, or subspecies?

It will be recalled that the water temperature of the normal habitat of *Daphnia* was 20°C and that it died when the temperature rose to 27°C . A mutant that required a temperature of 28°C appeared in the laboratory. It died at 20°C . However, if grown in a new geographic area with warmer temperature, this mutant would become the predominant type.

The ability of a species to mutate is its insurance against a changing environment. It represents a long range benefit.

Another isolating mechanism is geographic. If land or water geographic barriers are erected in a locality where a species is developing, different species or subspecies may be isolated on each side of the barrier. Some students of evolution feel this is perhaps the most important isolating mechanism. On the north and south banks of the Grand Canyon live two different species of squirrel, which must have come originally from one population. They do not interbreed because they do not cross the canyon.

Also, it is known that the jackrabbits on the north and south banks of the

Columbia River (in Washington and Oregon, respectively) show slightly different characteristics. No interbreeding is possible.

The barriers need not be as great as the Grand Canyon or the Columbia River. Perhaps an open field of grassland between two forests might serve as a break between plant and animal populations. The geographic barriers may vary in size from such strips of grassland to the immensity of the Grand Can-



Courtesy of Connecticut Agricultural Experiment Station, New Haven

FIG. 21-4 Cross sterility in maize. All the ears were open-pollinated. Those at left show usual results. The ears at right were selected against foreign pollen.

yon. If the barriers persist they may result in populations with different gene pools on opposite sides.

There are also "genetic barriers" of cross sterility. Although these are more pronounced for wide crosses where the chromosomes are incompatible, there are definite genic differences for cross sterility. I was impressed with an example of this one autumn (in 1931) when harvesting hand-pollinated ears of

corn in the genetic plot at the Connecticut Agricultural Experiment Station in New Haven. Many different genetic stocks were grown in a comparatively small field, and open-pollinated ears always had a great variety of colored kernels. It was surprising therefore to come across one row of plants in which nearly all of the open-pollinated kernels were white. The genotype was A/A c/c R/R Pr/Pr y/y Su/Su , a pearly white, flinty stock. Since c/c is not a common genotype in such a field, it frequently gets pollinated by C pollen, resulting in a colored aleurone. The unusual row had nearly all white kernels, which proved upon testing to have been self-pollinated. This white flint had a cross sterility gene, so that it discriminated against pollen from other types (Fig. 21-4). Here, then, was a genetic isolating mechanism. It is conceivable that such genes in a population could isolate one population from another, or separate a population in two, which could then go on without interbreeding and each develop its own gene pool.

CHROMOSOMAL ISOLATING MECHANISMS

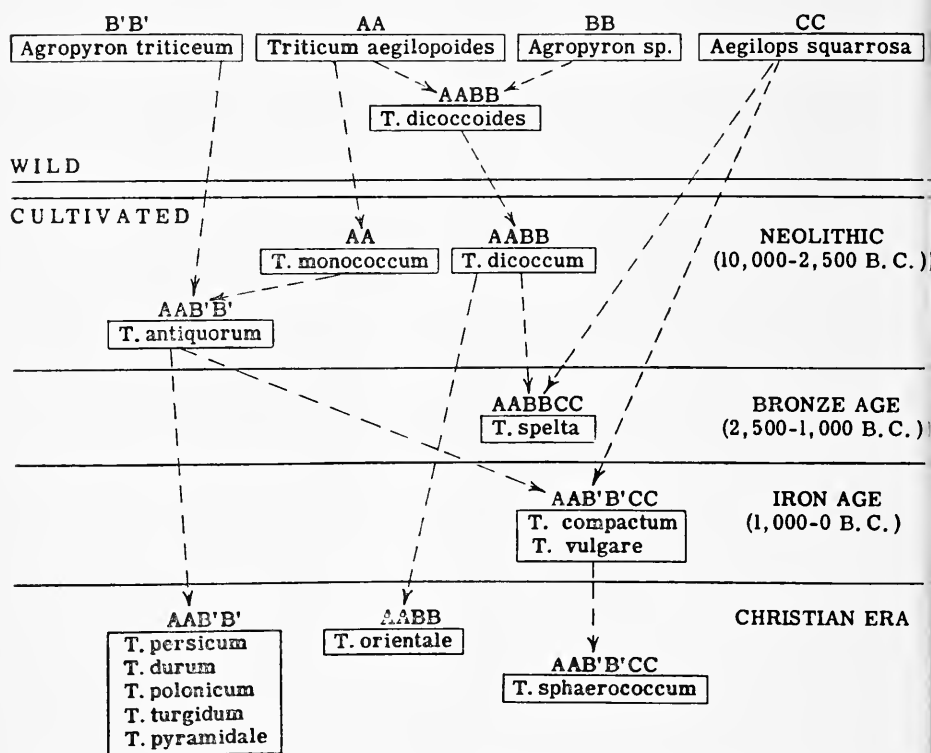
In different species of plants of a single genus, the chromosome numbers are commonly in multiples of some number. An example of this is wheat, cited in Chapter 16, in which the chromosomes are in multiples of seven, the basic number (n) for the most primitive species, *T. monococcum*. In *T. durum* and *T. dicoccum* $n = 14$, while in the bread wheats *T. vulgare* or *T. aestivum* $n = 21$. The bread wheats are not thought to be derived by a tripling of the number of *T. monococcum*. This would be autopolyploidy. Rather, wheat is thought to be derived from crosses of *T. monococcum* with a wild relative of wheat, *Aegilops* sp. ($n = 7$ also). See Fig. 21-5 for tentative derivatives and chronology of wheat.

AMPHIDIPOIDS

In the polyploid species in plants it is usually possible to make hybrids with closely related species having the same chromosome numbers and with those species whose numbers differ by not more than a single genome. Wider crosses are sometimes not possible, or if one can obtain an F_1 hybrid it may be highly sterile.

One example of this is the cabbage-radish hybrid of Karpechenko, an intergeneric hybrid, and another the amphidiploid *Nicotiana* hybrid, an interspecific cross found in East's laboratory in the mid-1920's. Cabbage, *Brassica oleracea*, and radish, *Raphanus sativus*, have the same chromosome numbers, $n = 9$. This does not mean that the two species can be crossed to give complete fertility. The two can be hybridized but the F_1 is almost completely sterile. The nine radish and nine cabbage chromosomes are not homologous, so that at meiosis in the F_1 there are 18 single chromosomes. In such a case the 18 chromosomes could assort at random at meiosis, so that potential

gametes could have from zero to 18 chromosomes. Apparently the 18 chromosome gametes are the only ones that function. Union of two such gametes produces an F_2 with just twice the chromosome number of the F_1 . Whereas the chromosomes in the F_1 are so different they do not pair at meiosis, each of the 18 chromosomes of the new amphidiploid has a homologous partner; pairing is normal or nearly so. Here then is essentially a new species, *Raphanobrassica*, completely fertile, produced in the laboratory by the hybridization of two different genera. This is known as an *allopolyploid* since it arose after the



Courtesy of Robert W. Schery; and Prentice Hall

FIG. 21-5 Derivation and chronology of wheat.

hybridization of two different types—in this case two genera. Chromosome doubling within the same species produces an autotetraploid, as discussed in Chapter 16.

The second example of amphidiploidy is one that arose in a species cross in *Nicotiana* (the genus of tobacco). *N. rustica*, $n = 24$, was pollinated by *N. paniculata*, $n = 12$. The reciprocal hybrid is not possible. As a general rule in making interspecific hybrids, success is possible only if the species with the higher chromosome number is used as the female parent. At meiosis in the F_1 , there were 12 pairs and 12 single chromosomes (Singleton 1928, 1932). Evi-

dently *N. rustica* and *N. paniculata* have one genome of 12 chromosomes in common. The 12 chromosomes of each species have a homologous partner in the F_1 , resulting in 12 pairs and leaving the 12 chromosomes (from another genome) of the *N. rustica* parent unpaired (Fig. 21-6).

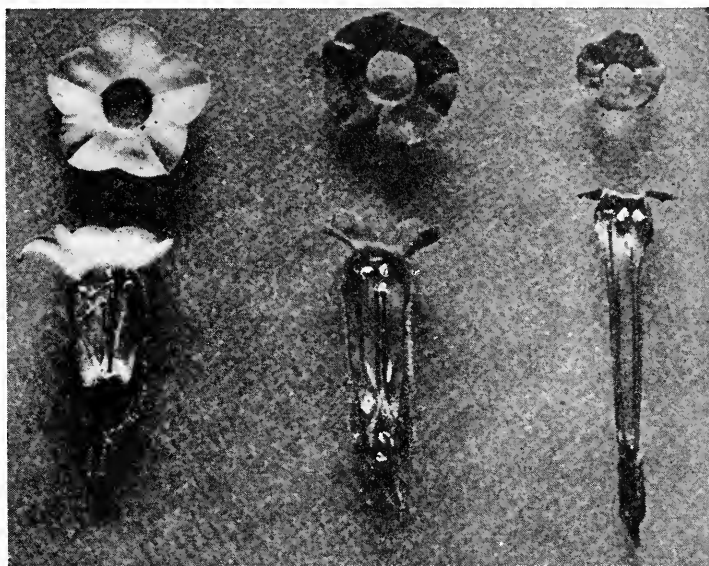


FIG. 21-6 Flowers of *N. rustica* (left), *N. paniculata* (right), and F_1 in center.

The F_1 bloomed profusely, but set very few seed capsules. After an unsuccessful attempt to obtain seed from greenhouse grown plants, many F_1 plants were grown in the field. They bloomed all summer without producing many seed capsules. Near the end of the flowering season several capsules were formed, so that several hundred seeds were obtained.

Upon examination of several plants of the F_2 it was indeed surprising to find that pairing was essentially normal, and there were 36 pairs, just twice the number of total chromosomes of the F_1 . Since there was normal pairing and gamete formation, the new species when self-pollinated had a high degree of fertility. It could be fertilized by *N. rustica*, but all attempts to secure F_1 plants by using *N. paniculata* pollen failed. Here, then, was a new species completely isolated genetically from one of its parents, *N. paniculata*. Genetic incompatibility is an efficient isolation mechanism of species.

AUTOPOLYPLOIDS

Since the advent of colchicine as an agent for chromosome doubling in species, the frequency of autopolyploids is much greater than found in nature. Autotetraploids usually differ from the diploids by having increased

leaf and flower size, also larger stomata, and in general a more luxuriant growth. A commercial application of autopolyploidy is found in the production of a seedless watermelon.

In the normal diploid melon *Citrullus vulgaris*, the $2n$ chromosome number



FIG. 21-7 Charles Darwin: a caricature from the year 1871, with the caption "Natural selection."

is 22. This number has been doubled by using colchicine to produce a tetraploid that has 44 chromosomes, just twice the number of the parent.

When the tetraploid is pollinated by a diploid, a triploid results. This triploid is sterile when pollinated by a diploid, producing no seeds. However, the diploid pollen stimulates fruit development. So, in the commercial production of seedless watermelons, the triploid hybrids are planted in a field with a normal diploid in every fourth or fifth row to serve as a pollinator. This results in fruit of excellent quality and few seeds (Fig. 21-8).

CONCLUDING REMARKS

Mutation is the basic building block of evolution. It is acted upon by natural selection, to get the new mutant gene in populations. The contribution of genetics to the understanding of evolution has been the thesis of this chapter. Darwin and Wallace put forth the natural selection theory before the science of genetics was established. Mendel's discovery of the gene and the many researches in this century on the nature of mutation have explained how evolution came about. The hereditary characters have as their determiners discrete particles known as genes. These mutate with known frequencies both forward and reverse.

The gene is the basic building block. The mortar that holds these bricks together is population genetics, developed largely in the last few decades. Combinations of mutant genes and an understanding of how they react in populations have given us the mechanism of organic evolution.

These mutant genes are located in the chromosomes, which also change in number, giving rise to polyploids and the formation of new species in this manner. Polyploids are more important in the plant species than in animals.

Changes in chromosome number are more a matter of the distribution of the genetic material available. But mutation is the process by which new genetic material is added to the gene pool of the population. It is the revelation of this process that has been important in our understanding of *how* evolution has taken place.

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Courtesy of O. J. Eigsti

FIG. 21-8 Seedless watermelons produced by crossing tetraploid and diploid to form triploid.

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PROBLEMS

- 21-1. Define or describe the following terms:

albino squirrel population	isolating mechanism
amphidiploid	mutant origin
autopolyploid	natural selection
gemmule	non-random mating
gene flow	origin of species
genetic equilibrium	pangensis
genetic isolation	population genetics
geographic isolation	selective mating
hemophilia	survival of fittest

- 21-2. Identify the following scientists, giving a major contribution with an approximate date.
- | | |
|-----------------|-----------------|
| Darwin, Charles | Muller, H. J. |
| East, E. M. | Reed, Sheldon |
| Levine, L. | Wallace, Alfred |
- 21-3. In a hypothetical population the forward mutation rate (f) was five times the reverse or back mutation rate. What would be the frequency of A (p) and that of a (q) when equilibrium was reached?
- 21-4. In Holstein dairy cattle, the usual color is black and white spotted. Occasionally a calf is born red and white. The red is recessive to black. Only black and white animals are used for breeding. Why is it that the gene for red has not been completely eliminated from the population?
- 21-5. If the frequency of the allele for red color (q) in Holsteins were .02, how often would a red and white calf be expected to appear, assuming mating to be at random? (Actually this is not a correct assumption, since man can manipulate the matings.)
- 21-6. How many generations would be required to reduce this frequency to .01? (For this problem assume that forward and back mutations cancelled each other, so this was not a factor.)
- 21-7. Why is the gene h^s (for sickling of the red blood cells) so prevalent in certain Negro populations when the homozygous condition h^s/h^s is nearly always lethal at an early age?
- 21-8. Why has polyploidy in the plant kingdom been of more evolutionary significance than in animals?

- 21-9. Recently strains of staphylococcus resistant to penicillin have appeared in hospitals following repeated use of penicillin as an antibiotic. Discuss the evolutionary aspect of the appearance of this resistant strain. Is this the inheritance of an acquired character?
- 21-10. In survival of the fittest, what is meant by fit?

The Nature of the Gene

BEFORE PRESENTING our current understanding of the nature of the gene, let us review briefly the different steps that have led to this knowledge.

IN THE BEGINNING, THE GENE

It is quite appropriate that the hereditary determiner is named the gene, derived from the Greek word *genesis*, meaning to be born. The more we learn about the gene and how it functions and reduplicates itself, the more it seems possible that the gene is the basic unit of life, capable of self-reproduction, which is the prime criterion.

The science of genetics began when it was realized that hereditary traits had basic elementary particles as their determiners. This theory, postulated by Mendel, was what made his proposal so different from that of his predecessors, who had considerable knowledge about heredity. His determiner he called the element, later named gene by Johannsen.

For 35 years (until 1900) after Mendel presented his findings, the science of genetics did not advance, largely because Mendel's contemporaries were not ready for so startling a discovery. It was only after the researches of Correns, De Vries, and Tschermak that the present science was established. Curiously enough, this field of study might have developed as rapidly as it did if Mendel had never lived. All three of the later investigators reached the same conclusions as Mendel. They came upon his paper only after they had each separately and independently made a new and startling discovery, or so they thought.

Our knowledge of the gene and the way it can be distributed in future generations advanced rapidly in the early decades of the present century. Morgan's explanation of linkage postulated that the genes were arranged linearly

in the chromosomes, a big step forward in the understanding of the relationship of genes and chromosomes. Following this came the "beads on the string" explanation of genes. Although somewhat naive, it did help to clarify the mechanics of gene separation when several genes were linked in the same chromosome.

GENES AND MUTAGENS

Spontaneous mutations are rare events and their origin is not subject to experimental study. It was only after mutagens became available for increasing the frequency of such events that progress was made in our understanding of how the gene works. The first mutagen was ionizing radiation from either radium or X rays. Roentgen's discovery of X rays followed on the heels of Weismann's propounding of the germ plasm theory, which conceived of the germ plasm as being immutable by environmental conditions. Weismann's theory did not reckon with a change in the environment in the nature of penetrating radiation that could pierce through the internal cellular structure and change the hereditary particles in the nucleus. Early in the present century, many experiments were made on the effects of radiation on seeds and plants. Gager was a pioneer in this work. It was not until the middle 1920's that radiation was used so successfully in producing gene changes. This was the work of Muller and of Stadler. Somewhat later, during World War II, the use of chemical mutagens was pioneered by Auerbach in Scotland.

GENETIC MUTAGENS

During all this time a considerable body of evidence was accumulating that certain genes could cause mutations. One of these was the dotted gene, *Dt*, in maize, which causes the gene *a* to mutate to *A*. The gene *A* is a basic one, necessary for anthocyanin production in the kernel and in the plant. A homozygous recessive stock *a/a* in the presence of *Dt* will mutate to *A* giving dots of anthocyanin color in the kernel or pigmented sectors in the leaves. The *Dt* gene is located near the end of the short arm of chromosome 9, while the *a* gene is in chromosome 3. The effect is produced even though the *Dt* is in a separate chromosome from *a*.

Another genetic mutagen is that found by McClintock, labeled the *Ac—Ds* system. The *Ac* indicates an activator and the *Ds* dissociation that produces chromosome breakage. The activator stimulates the *Ds* to produce chromosome breaks. In the presence of *Ac* in the same cell, the *Ds* locus produces chromosome breaks whose results can be studied in stocks well marked genetically. One of the most thoroughly investigated by McClintock is the *c* locus. The phenotypic expression of the *Ac—Ds* system acting at this locus is mottled kernels. These appear as a result of the repeated breaks at the *Ds* locus. The *Ds* element is thought to be a piece of heterochromatin.

POSITION EFFECT IN DROSOPHILA

A type of position effect in which genes in the *cis* position produce a different phenotypic expression than in the *trans* position was noted in Chapter 14. A position effect also occurs sometimes when the genes in the euchromatin are rearranged in such a way that they are next to heterochromatin. By X ray it is possible to break chromosomes and change their architecture, as pointed out in Chapter 16.

The X chromosome can be broken near the locus of the gene for white and translocated to the heterochromatic region of either the second, third, or fourth chromosomes. Then the normal + allele of white (red eyes) in the X chromosome is adjacent to the heterochromatin of the other chromosome. In this new location the gene for red is in a new "environment." Such a fly does not have normal red eyes, but white eyes with patches of red, commonly known as white-mottled. Following another chromosome breakage, it is possible to restore the wild-type allele (+) to a site adjacent to euchromatin. The red phenotype is restored, which shows that the location next to heterochromatin produces the mottling.

PARAMUTATION IN MAIZE

One of the basic tenets of Mendel's law is that genes segregating from a hybrid are in no way affected by a temporary association with a different allele in the homologous chromosome. In practically all organisms studied, this rule holds. A rare exception has been found by R. A. Brink for the *R* locus in maize (Brink 1957).

In testcrosses of different *R* alleles as the male parent, it was found that R^r ♂ gametes (self-colored aleurone) produced by R^r/R^{st} plants (heterozygous for stippled aleurone) were found to give a different phenotype than the R^r gametes produced by R^r/R^r plants. The kernels were nearly all of the type that would have been expected from a pollination of r/r by R^{st}/R^{st} . In some way the R^{st} allele had influenced the color produced by the R^r allele. The altered form of R^r in the R^r/R^r testcross progeny is transmitted through the succeeding sporophyte generation and, therefore, is heritable.

Detailed evidence for this unusual inheritance can be found in the references at the end of the chapter. It represents an interesting exception to the rule that alleles segregate out of a hybrid unaffected by their temporary association as homologues of alleles of a different kind. It is not known how widespread this phenomenon is, but thousands of instances of normal behavior had been observed before the ingenious analysis by Brink.

SUBDIVISIONS OF THE GENE

As our understanding of the gene has increased, it has become evident that the gene is capable of subdivision. In a way, this is similar to the

atom, which gets its name from the Greek word *atomos* meaning uncut or indivisible. Yet any high school graduate who has had a course in physics knows the atom is composed of three primary particles, the proton and neutron in the nucleus, with the electron whirling around outside.

The locus of a known gene in the chromosome has been shown to be much more complex than at first believed. Multiple alleles have been found to be pseudoalleles with certain fixed amounts of crossing-over. It seems reasonable to assume that the different pseudoalleles have arisen from a common gene by duplication and by mistakes in crossing-over. It is a characteristic of all multiple alleles and pseudoalleles that they affect similar physiological and chemical functions. This is evidence that suggests they arose from a common "ancestor."

Genetic fine structure of a number of genes has been established, in viruses and even in the higher plants. In maize the waxy locus has been carefully analyzed by O. E. Nelson, who hybridized two different waxy strains and examined millions of pollen grains. He was studying the frequency of normal pollen grains (blue staining with iodine) in a field of predominantly waxy grains (red staining). These blue grains could represent back mutations from $wx \rightarrow Wx$, or they could be crossovers within the compound waxy locus. The frequency of Wx grains was considered too great to represent back mutations from $wx \rightarrow Wx$. The most plausible theory to explain the wx grains is that the wx locus is compound and the wx grains really represent crossovers within this locus. Nelson found that different strains of the waxy gene gave different frequencies of reversions to normal Wx . By comparing the frequency of reversion when different stocks were crossed, Nelson was able to construct a map of the waxy locus showing the relative distance of the different waxy "sub-genes" from each other.

A new terminology has arisen to describe the subdivisions of the gene. Benzer (1957) has proposed three different terms as follows:

1. A *muton* is the smallest element capable of giving rise to a new form by mutation.
2. A *recon* is the smallest indivisible unit that gives rise to new forms by recombination.
3. A *cistron* is the region within which all mutants show a *cis-trans* position effect, i.e., they are not complementary as are mutants located at different loci.

According to these definitions the cistron is the largest of the three, with the recon second, and the muton smallest of all. The term cistron would be more or less synonymous with the hereditary particle we have known so long as the gene. Mutons and recons are subdivisions.

THE NATURE OF THE GENE

This has been an object of study and speculation almost as long as we have known of genes. There is no doubt that most hereditary characters are determined by particles (genes) in the chromosomes, rather than in the cytoplasm. It is also known that the chief chemical constituent of the chromosome is deoxyribonucleic (DNA). This is constant in amount from cell to cell in diploid tissue. Sperm cells (haploid) have only half as much.

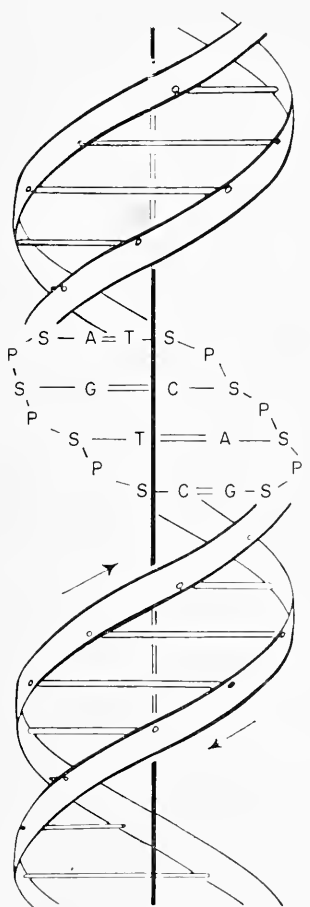
Although the nucleus contains other substances such as proteins, only the DNA is positively correlated with critical hereditary material. The proteins and other substances do not exhibit the constancy of distribution from cell to cell as does the DNA.

Another bit of evidence linking DNA with hereditary material is that the absorption spectrum of nucleic acid is similar to the spectrum of ultraviolet light that is efficient in producing mutations. Also the transforming principle (Chapter 19) is DNA. Altogether, many lines of evidence pointed to DNA as the hereditary material before the structure of DNA was known.

STRUCTURE OF THE DNA MOLECULE

In 1953 J. D. Watson and F. H. C. Crick proposed a model for the structure of the DNA molecule. This gave for the first time a possible structure for a self-duplicating molecule. According to this hypothesis, DNA is composed of a double-stranded helix with the two polynucleotide strands bonded together through complementary purine and pyrimidine bases (Fig. 22-1). This model is satisfying biologically. It provides a mechanism for self-duplication, which is fundamental for cell division, reproduction,

and growth of organisms. The genes and the chromosomes are duplicated faithfully at each cell division. Before cell division, the two halves of the DNA molecule split at the location of the double hydrogen bond and then each half



Courtesy of G. W. Beadle;
and Oregon University Press

FIG. 22-1 Watson-Crick model of DNA molecule, from a drawing by Beadle.

of the molecule proceeds to build a new half to replace the one from which it has separated (Fig. 22-2).

There are three fundamental parts to the DNA molecule which give it the appearance of a double circular stair case. The two sides of the stairway are analogous to the two sides of the molecule. These are made of alternate phosphate and deoxyribose sugar components. The purines and pyrimidines represent the steps of the stairs. The two purines involved are adenine and guanine, and the two pyrimidines are thymine and cytosine, shown in the diagram as A, G, T, and C. The two dots stand for the hydrogen bonds.

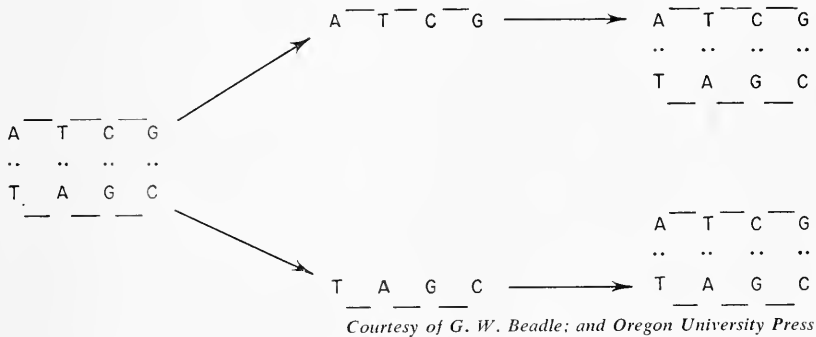


FIG. 22-2 Replication and division of DNA molecule.

The four bases A, G, T, and C are not arranged in a random fashion, but in an orderly manner with A and T opposite each other and C and G opposite each other. These base pairs are thought to carry all the genetic specifications as to what an organism is and how it develops. These specifications are sometimes called genetic information. It is as though we had an alphabet of four letters in which are coded all the genetic specifications of all living things. The number of these base pairs that make up a given functional unit of genetic specifications, the gene, is not known. Beadle has estimated 1000 to 10,000. To be conservative, let us assume it is 1000. Since there are four base pairs in each unit and there are 1000 such units, the number of ways these can be arranged is 4^{1000} . This is a tremendous number—in fact it is incomprehensible. You will recall that in Chapter 12, for another purpose, we calculated how large a corn field must be to accommodate 4^{30} plants. This fantastic corn field, at conventional spacing, turned out to be 2000 times the total land area of the earth. And 4^{30} is infinitesimally small in comparison with 4^{1000} . The number given represents the possibilities for a single gene. How many specifications can there be in a single submicroscopic virus or in a microscopic cell nucleus? Beadle has estimated that in a bacterial virus there are present something like 20,000 turns of the DNA helix. Each turn consists of 10 base pairs. This would mean 200,000 base pairs. Thus the DNA of a single virus could be put together $4^{200,000}$ ways. The nucleus of a cell of man contains about 1000 times

as much DNA as that of the virus. In man there would be $4^{200,000,000}$ ways of building this amount of DNA.

So it seems that the four-letter code is sufficient for the genetic specifications.

This is one of the satisfying features of the Watson-Crick model. Not only does it provide a mechanism for self-duplication, but it contains a workable formula for presenting the varied genetic specifications required for any organism. Base pairs will soon become as familiar a part of genetic language as the gene. The fact to remember is that the two purine bases adenine and guanine always pair with one of the pyrimidine bases, thymine and cytosine.

GENE MUTATION ACCORDING TO THE WATSON-CRICK MODEL OF DNA

Gene mutations represent changes in the base pairs. These alterations are listed by Beadle as being of four types: substitution, transposition, omission, and duplication. In each of these types, one or more base pairs could be involved. The exact mechanism by which these changes occur is not known and is a problem of future research. Already the Watson-Crick model has served as a stimulus to geneticists and biochemists alike.

Some of the viruses consist primarily of DNA and are sometimes referred to as naked genes. In the evolutionary scale it seems likely that something similar to a virus was the first living thing and that all other organisms arose using the genes as basic building blocks. This is comparable to the evolution of the chemical elements, in which the basic building block is hydrogen. All other elements have been derived from it.

ROLE OF RNA

Ribonucleic acid (RNA) is present mainly in the cytoplasm, and in the nucleolus, with a small amount in the chromosomes. The tobacco mosaic virus consists almost entirely of RNA, showing that RNA is capable of the transmission of hereditary characters. The tobacco mosaic virus is on the border between the living and non-living. It can be crystallized and kept in containers on the shelves apparently indefinitely. When it is dissolved and injected into tobacco plants, it multiplies and has all characteristics of a living particle.

The structure of RNA is not as well known as DNA, so we are not certain how it duplicates itself. In all cases of DNA or RNA duplication, the materials present in the protoplasm of the cell are used in the duplication process. The RNA and DNA are dependent upon substances in the host cell for their multiplication.

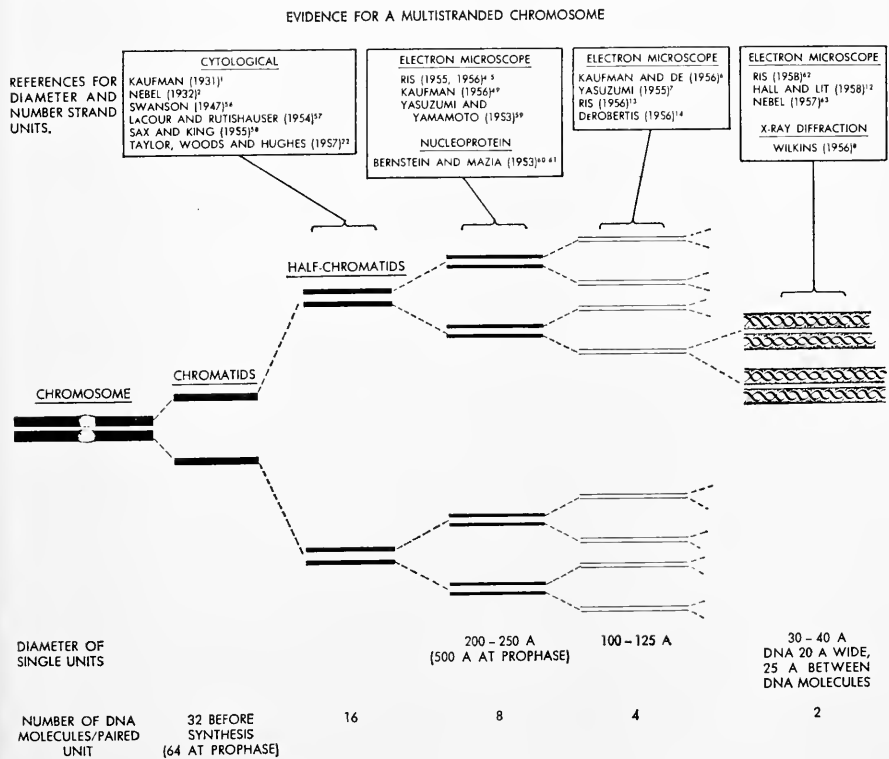
Another possible role of RNA is the translation of the genetic specifications, or blueprints found in the DNA, into action and the production of the final metabolic products. Since some viruses contain only RNA and protein, with

no DNA, it seems plausible that the code in the DNA might be translated into action through RNA.

DNA AND STRANDS OF CHROMOSOMES

It is not fully understood just what is the relationship between the strands of DNA molecule and the strands of chromosomes. This is one of the exciting areas of research, and undoubtedly this relationship will be explained in the future. An interesting theory as to what this relationship might be was presented by Dale Steffensen to a symposium on the Structure and Function of Genic Material at the Brookhaven National Laboratory in 1959. The evidence for a multi-stranded chromosome is presented. A pair of chromosomes as originally seen under the microscope actually may contain as many as 64 strands, which could represent strands of the DNA molecule (Fig. 22-3).

This drawing shows the subdivision into the different numbers of strands. It also cites the authorities responsible for each step of the process. The half chromatid subdivision into four strands is based upon cytological evidence.



Courtesy of Dale Steffensen; and Brookhaven National Laboratory

FIG. 22-3 Possible relationship between strands of the chromosomes and of the DNA molecule.

The division into eight strands is based upon evidence from the electron microscope, as is the subdivision into 16 strands. Each of the 16 strands is thought to be made up of four strands of the DNA molecules, making 64 strands in all. Many unknowns remain to be resolved in this scheme. However, it does provide a method for bringing together the information about chromosomes and the DNA molecule into a workable hypothesis for explaining the physical-chemical basis of heredity.

Thus we have traveled a long way from the original gene concept of Mendel to the structure of the DNA molecule and how the gene is duplicated and passed on from generation to generation. In closing, we wish to quote from R. B. Goldschmidt, who was a leader in the formative years of genetics. This is the conclusion of a talk prepared for the Golden Jubilee of the Science of Genetics, which was held in 1950.

We come to the end of this admittedly incomplete record of the growth of genetics in the first half century of its existence. Looking back at the history of thought we realize that from time to time discoveries are made and ideas proposed which, though starting in an apparently limited field, at once illuminate all fields of human knowledge and force them to adapt their mode of thinking to the new insight. In the modern history of science such an event was Galileo's experimental work which started the new era of inductive science. Another one was the establishment of the theory of evolution by Darwin. Accepting the risk of being reproached with carrying too far the spirit of the Jubilee, I state that the rise of genetics is another instance of discovery of new facts and of the conception of new ideas which have deeply influenced many fields of intellectual endeavor and the end is not yet in sight. Taxonomy and comparative anatomy, embryology and cytology, physiology and ecology have felt the impact. Evolutionary thought has been rejuvenated; paleontology has been forced to reconsider its basic tenets; zoology and botany have been welded together; eugenics and anthropology were given a solid basis; psychology and sociology have begun to take notice; biochemistry has not only been assimilated as a tool, but has received important stimulus in return. Medicine has been deeply influenced; bacteriology owes to genetics a new upsurge; and virology has assumed added importance; the philosophy of the organism is reconsidered and theoretical physics begins to introduce genetics into its most subtle deliberations. Geneticists may take reasonable pride in such performance and still more in the fact that progress has not slackened its pace and that the future holds no limits to further conquests.

More than four hundred years ago one of the most colorful figures of the Age of Reformation, Ulrich von Hutten, overwhelmed by the happenings of his day and the hope of a still greater future, exclaimed the famous words "*Es ist eine Lust zu leben*" ("It is a joy to be alive"). May I adopt this exclamation for the status of genetics today and close with the words: "Geneticists of 1950, *Es ist eine Lust zu leben!*"

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PROBLEMS

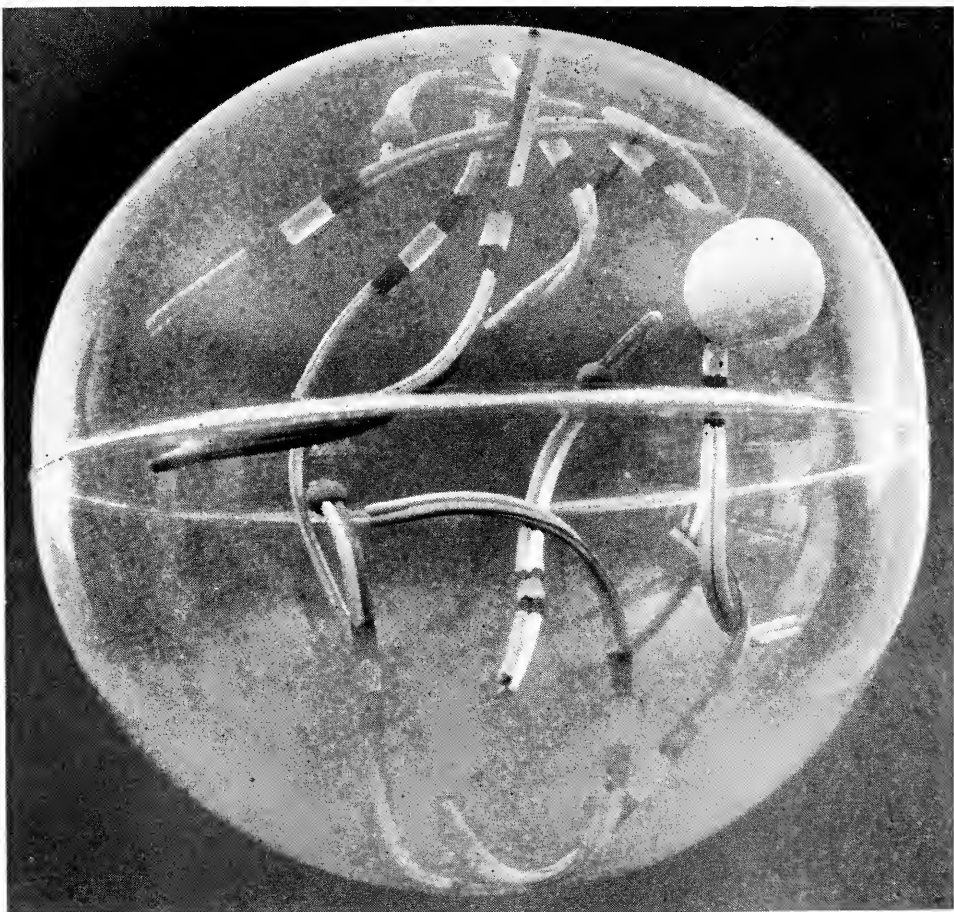
22-1. Define or describe the following:

<i>Ac-Ds</i> system maize	muton
changes in base pairs—mutation	paramutation
substitution	position effect
transposition	purines
omission	adenine
duplication	guanine
<i>cis</i> position—pseudoallele	pyrimidines
cistron	thymine
DNA	cytosine
<i>Dt</i> gene (maize)	recon
genetic fine structure	RNA, role of
genetic mutagen	subdivision of gene
multi-stranded chromosome	<i>trans</i> position—pseudoallele
mutagen	

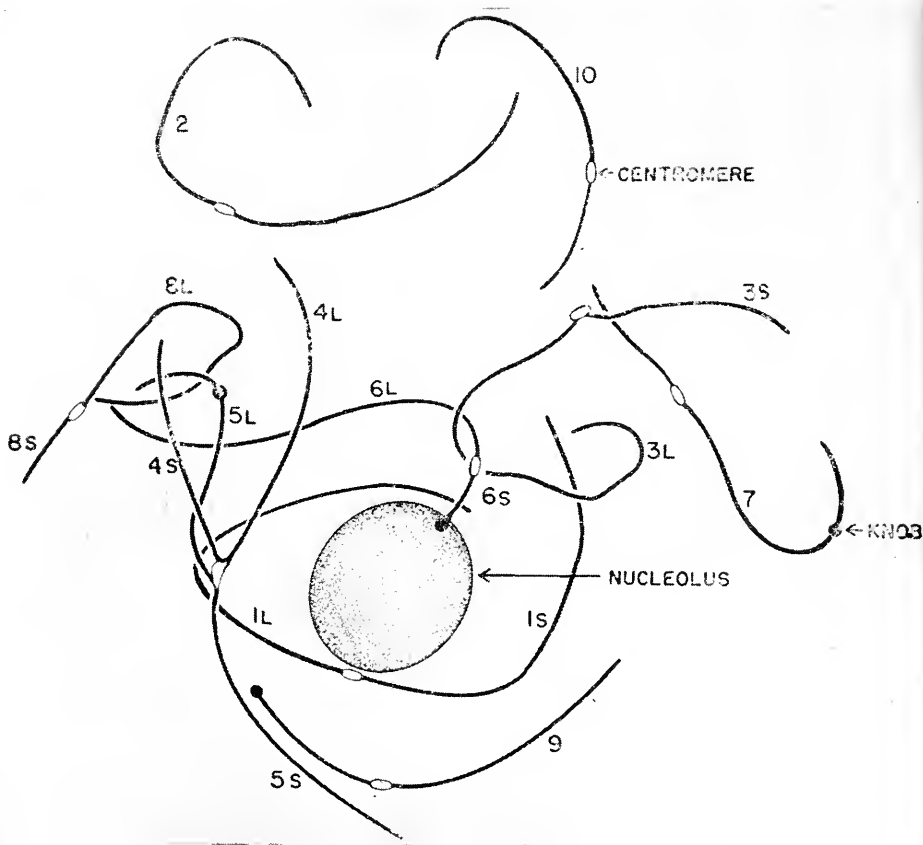
22-2. Identify the following scientists, giving a major contribution with an approximate date.

Auerbach, C.	McClintock, B
Beadle, G. W.	Muller, H. J.
Benzer, S.	Nelson, O. E.
Brink, R. A.	Stadler, L. J.
Crick, F. H. C.	Steffensen, Dale
Goldschmidt, R. B.	Watson, J. D.

Appendix A—The Ten Chromosomes of Maize*



* Reproduced by permission from *The Ten Chromosomes of Maize* by the Research Department of the DeKalb Agricultural Association, Inc., of DeKalb, Illinois. The author is indebted to the kindness of the association and in particular to Loring M. Jones of the Research Department.



Courtesy M. M. Rhoades

Schematic diagram of pachytene chromosomes. Each bivalent chromosome is represented by a single line in order to simplify the diagram.



Photomicrograph at left shows actual maize (haploid) chromosomes at the metaphase stage of cell division in mitosis.

CHROMOSOME I

Chromosome I is the longest of the maize chromosomes. It carries genes for grasshopper resistance and leaf blight resistance (*Helminthosporium carbonum*).

Locus 0 sr_1 Striate - 1

Leaves have fine, longitudinal white stripes which persist throughout the life of the plant.

Locus 14 ag Resistance to grasshoppers

A South American variety, *Maize amargo*, carries this gene.

Locus 15 ga_6 Gametophyte factor - 6

Pollen with ga is eliminated when carried by the male gamete.

Locus 23 zb_4 Zebra striped - 4

Seedling has alternating chlorotic cross bands on leaves which disappear as plant matures.

Locus 25 ms_{17} Male sterile - 17

Anthers do not usually exert. Some pollen grains shed occasionally.



sr_1 Striate - 1

zb_4 Zebra striped - 4





ts₂ Tassel seed - 2

Locus 27 *ts₂* Tassel seed - 2

The terminal inflorescence is usually completely pistillate. An ear will develop if terminal inflorescence is removed after emergence. Secondary florets in ear develop which give an irregular arrangement of kernels.

Locus 28 *P* Pericarp and cob color

A large series of alleles exists for pericarp and cob color. Example: *P^{rr}* has red pericarp and cob color.

Locus 30 *zl* Zygotic lethal

This lethal character kills the young sporophyte and endosperm.

Locus 55 *as* Asynaptic

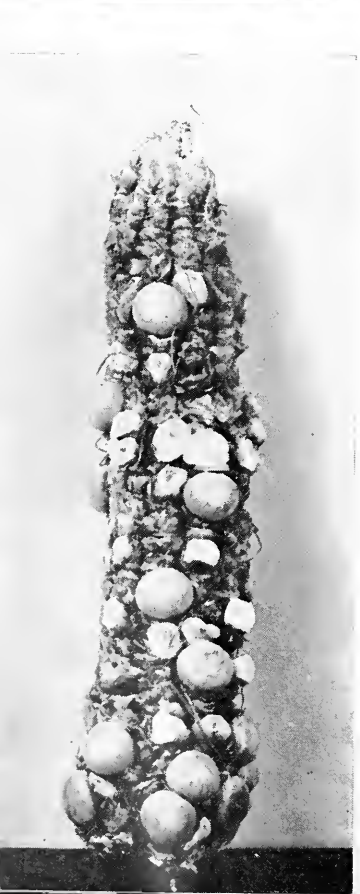
Plants are partially sterile due to a lack of association of homologous chromosomes during the first meiotic division. Usually does not shed pollen but will set a few kernels when pollinated by normal plants.

Locus 58 *pa* Pollen abortion

This gene transmitted through the female only.

Locus 78 *hm* Susceptible to leaf blight, *Helminthosporium carbonum*

hm Susceptible to *H. carbonum*



as Asynaptic



Locus 92 br_1 Brachytic - 1

Stalk internodes shortened, especially those below the ear. Other parts of the plant not reduced.

Locus 96 Vg Vestigial glume

Glume length in the tassel and ear is greatly reduced. Anthers in the tassel are completely exposed. Some pollen shed occasionally.

Locus 97 f_1 Fine stripe - 1

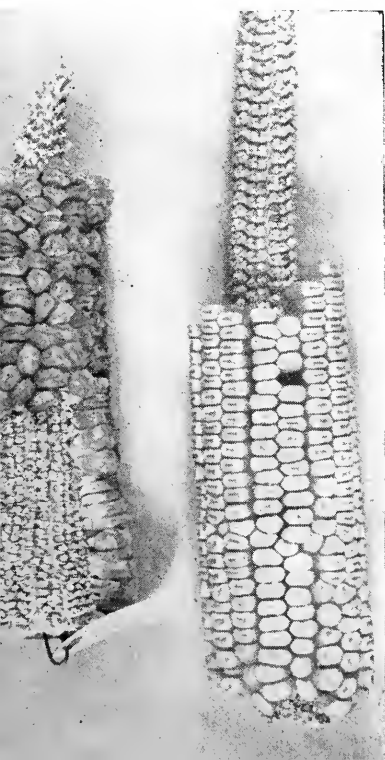
Seedling virescent. Leaf blades have fine stripes of white tissues.



br_1 Brachytic - 1



Portion of tassel showing Vg



f_1 Fine stripe - 1





*an*₁ Anther ear - 1



Kn Knotted leaf

Locus *ad* Adherent

Locus 114 *an*₁ Anther ear - 1

Stamens develop throughout pistillate inflorescence. Ear terminates with an unbranched spike consisting of staminate inflorescence.

Locus 126 *Ts*₃ Tassel seed - 3

Similar to *ts*₂, Locus 27, except that the terminal inflorescence consists of both staminate and pistillate components.

Locus 136 *Kn* Knotted leaf

Proliferation of vascular tissues results in a kinked or knotted appearance.

Locus 145 *gs*₁ Green striped - 1

Dull green stripes show between vascular tissues at the third or fourth leaf stage and later.

ad Adherent (at right) normal (left)



Ts₆ Tassel seed - 6

Locus 166 *Ts₆* Tassel seed - 6

See *Ts₃*, locus 126, on this chromosome.

Locus 172 *bm₂* Brown midrib - 2

A brown plant color develops in the midrib and vascular tissue bundles of leaf blade and sheath. Less intense than *bm₁* on Chromosome V.



CHROMOSOME II

Chromosome II has one of the basic plant color genes, *B*. In combination with *A₁*, *A₂*, *Pl*, *R^{gs}* plants will be purple with green anthers.

Locus 0 *ws₃* White sheath - 3

Leaf sheaths light yellow in color due to absence of chlorophyll.

Locus 4 *al* Albescens

Plants usually green at seedling stage, later becoming whitish. Visibility variable, oftentimes poor.

Locus 11 *lg₁* Liguleless leaf - 1

Leaves lack ligule as well as auricle and stand upright at base.

Locus 30 *gl₂* Glossy seedling - 2

Distinguishable at the seedling stage when leaves have glossy appearance in bright light.

gl₂ Glossy seedling - 2



lg₁ Liguleless leaf - 1





sk Silkless

Locus 49 *B* Plant color booster

Gives intense sun red, purple, or brown plant color in appropriate genotypes.

Locus 54 *gs₂* Green striped - 2

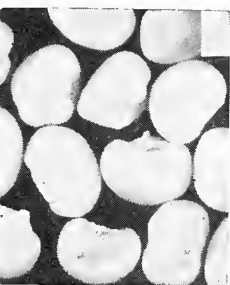
Mature plants have green stripes similar to *gs₁*, locus 145, Chromosome I.

Locus 56 *sk* Silkless

Plants are female sterile from pistillate abortion. Cobs are normal in growth and contain many anthers.

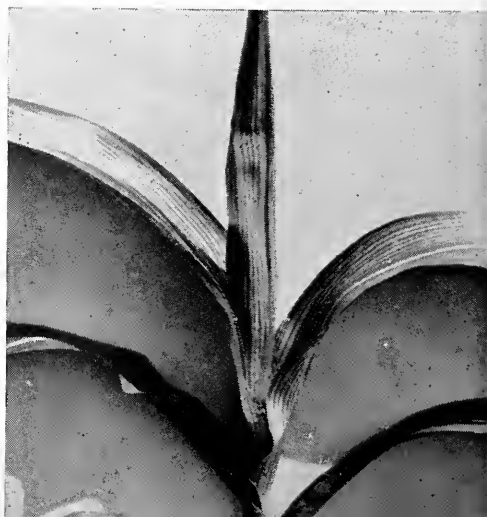
Locus 68 *fl₁* Floury endosperm

Endosperm consists of non-corneous starch. *fl fl* combinations give a floury endosperm whereas *Fl Fl fl* are flinty.



At left: Ear and kernels showing *fl*, Floury endosperm.

gs₂ Green striped - 2





v_4 Virescent - 4 (left)



ts_1 Tassel seed - 1

Locus 74 ts_1 Tassel seed - 1

Similar to ts_2 , Chromosome I. Ears develop if terminal inflorescence is removed soon after emergence.

Locus di Disintegrated endosperm

Locus 82 v_4 Virescent - 4

Seedlings are yellowish green. Plants turn green very slowly. Can be distinguished more readily at later stages than other virescents.

Locus 124 Ch Chocolate pericarp

Outer seed coat is chocolate or dark brown in color.



**Rf Fertility restoration
(Chromosome III)**

CHROMOSOME III

The long arm of this chromosome contains one of the genes for corn borer resistance (5). Other genes for corn borer resistance are found on the long arms of Chromosomes IV and V. Chromosome III also has a gene for fertility restoration (6).



*cr*₁ Crinkly leaf - 1

Locus 0 *cr*₁ Crinkly leaf - 1

Plants somewhat shorter than normal. Leaves have characteristic crinkled appearance at the base.

Locus 18 *d*₁ Dwarf plant - 1

Plants very short with broad thick leaves. Stamens develop on the ears. Staminate inflorescence is compact.

Locus 26 *ra*₂ Ramosa ear - 2

Ear branched at base. Tassel is branched and conical.

Locus 31 *Cg* Corn grass

Leaves narrow, many tillers. Pistillate inflorescence in axil of the leaves, not on a well defined ear. (7)

Locus 40 *rt* Rootless

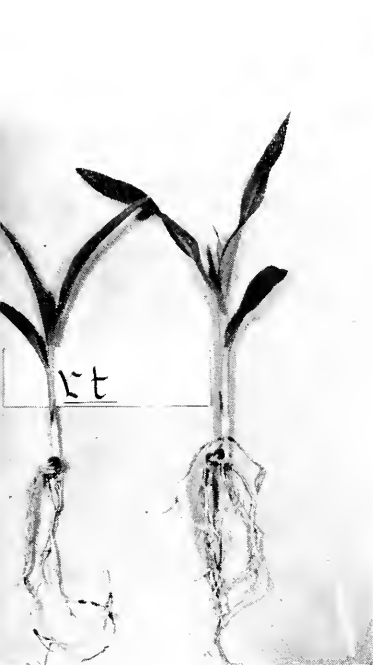
Plants have very few roots.



*d*₁ Dwarf plant - 1

*ra*₂ Ramosa ear - 2 (tassel)





rt Rootless (at left)
normal seedling at the right.



Cg Corn grass - pistillate inflorescence

Cg Corn grass



ra₂ Ramosa ear - 2





*Lg*₃ Liguleless leaf - 3

Locus 46 *Lg*₃ Liguleless leaf - 3

Has only a portion of the ligule present.

Locus 48 *Rg* Ragged leaf

Weak plants with split and torn leaves. This gene expression occurs when plants are half grown.

Locus — *Rf* Fertility restoration

Locus 55 *ts*₄ Tassel seed - 4

A few kernels are usually produced in the tassel. Both pistillate and staminate inflorescence develops in the tassel. Pollen is shed.

Locus 72 *ba*₁ Barren stalk - 1

No ear shoot develops. These plants are female sterile. Stalks lack concave depression at ear node.

Locus 78 *lg*₂ Liguleless leaf - 2

Similar to *lg*₁, Chromosome II, except that a ligule may be present on a few leaves.

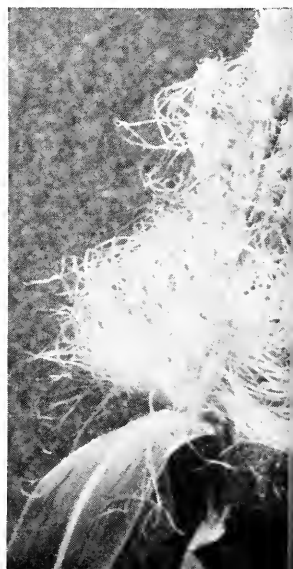
Locus 83 *na*₁ Nana dwarf - 1

Plants are one-fourth to one-third normal height. Leaves are short, stiff and twisted.



Rg Ragged leaf

*ts*₄ Tassel seed - 4





ba_1 Barren stalk - 1



sh_2 Shrunk endosperm - 2

Locus 111 A_1^b Brown pericarp

The A_1 gene gives plant, aleurone and pericarp color.

Locus 112 sh_2 Shrunk endosperm - 2

Endosperm shrinks greatly at maturity leaving a hollow crown and often shrunken sides on the kernel. (See sh_1 on Chromosome IX).

l_2 Liguleless leaf - 2



na_1 Nana dwarf - 1





d_2 Dwarf plant - 2
(Locus unknown on Chromosome III)



et Etched endosperm

Locus 123 *et* Etched endosperm

Endosperm has scarred appearance. Seedlings are virescent.

Locus 129 ga_7 Gametophyte factor - 7

Acts similar to *Ga* on Chromosome IV but does so independently of the genetic constitution of the silks.

CHROMOSOME IV

This chromosome has two genes for resistance to *Helminthosporium turcicum* leaf blight, one in the upper arm and one in the lower arm. (8)

Locus 0 de_1 Defective endosperm - 1

Endosperm does not develop completely although this locus, de_1 , comes closer to normal development than others.

Locus 35 *Ga* Gametophyte factor

Ga pollen in competition with *ga* pollen on *Ga* silks produces from 95 to 99% of the kernels.



Locus 55 *st* Sticky chromosome

Plants are partially female sterile and usually completely male sterile. Meiotic divisions are irregular. Classification good by striate leaves.

Locus 56 Ts_5 Tassel seed - 5

A few kernels develop in the tassel. Secondary florets develop in the ears. Tassels are not as compact as in ts_4 , Chromosome III.

Ts_5 Tassel seed - 5

la Lazy



Locus 60 *la* Lazy

Plants develop normally except they tend to grow earthward rather than upright. Stalks of "lazy" plants are as strong as normal upright plants.

Locus 66 *sp* Small pollen

Pollen development normal but size is smaller. This characteristic is transmitted through the ovule.

Locus 71 *su*₁ Sugary endosperm - 1

Endosperm translucent and wrinkled at maturity.

Locus 73 *lo* Lethal ovule

Ovules abort. This gene is transmitted almost entirely by the pollen.

Locus 75 *de*₁₆ Defective endosperm - 16

Endosperm does not develop completely. This is a lethal character.

Locus 84 *zb*₆ Zebra striped - 6

Leaves of almost mature corn have chlorotic cross bands.

Locus 86 *gl*₁ Glossy seedling - 4

Similar to *gl*₇ on Chromosome II and *gl*₁ on Chromosome VII.

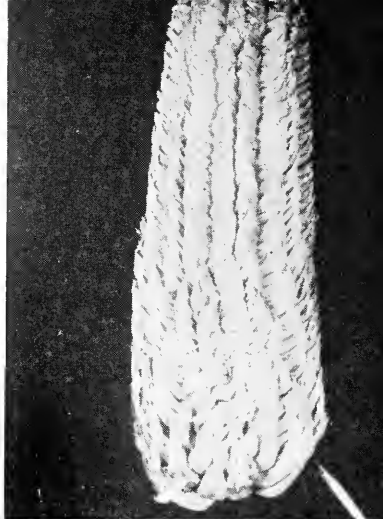
*su*₁ Sugary endosperm - 1





Tu Tunicate
Pistillate
inflorescence

Tu Tunicate
Staminate
inflorescence



Locus 107 *Tu* Tunicate

Glumes are long in both staminate and pistillate inflorescence. Individual kernels are more or less completely enclosed. The homozygote, *TuTu*, is usually male sterile.

Locus 112 *j*₂ Japonica - 2

Mature plant has variegated white striping. Seedlings nearly all white. Leaves usually narrow and irregular.

Locus 118 *gl*₃ Glossy seedling - 3

Young leaves have "glossy" surface. When immersed in water non-glossy, *Gl*₃, seedlings have metallic sheen.



*j*₂ Japonica - 2

CHROMOSOME V

Between loci 14 and 21 is found the gene *ae*, amylose extender. (9) This locus is important for the production of corn with a high amylose starch content.

Locus 0 *gl*₁₇ Glossy seedling - 17

See other *gl* loci on Chromosomes II, IV and VII.

Locus 1 *A*₂ Anthocyanin - 2

The dominant allelomorph is complementary to *A*₁*a*₁, locus 111, Chromosome III, in the production of plant and aleurone color.

Locus 6 *bm*₁ Brown midrib - 1

Brown color develops over vascular tissue of leaf blade, leaf sheath, stalk and roots.

Locus 8 *bt*₁ Brittle endosperm - 1

Endosperm more or less translucent, often wrinkled and greatly shrunken.

Locus 10 *v*₃ Virescent seedling - 3

Seedlings are light yellow but soon turn to green.

Locus 12 *bv* Brevis

Plants about one half normal height due primarily to shortened internodes in the region of pistillate inflorescence.

Locus 31 *pr* Red aleurone

Together with genes for aleurone and scutellum color, *pr* gives red aleurone. The dominant allele, *Pr*, gives purple aleurone.



ae Amylose extender



*bt*₁ Brittle endosperm - 1

bv Brevis





v_2 Virescent seedling at right — normal left



su_2 Sugary endosperm

py Pygmy seedling at left



Locus 40 ys_1 Yellow stripe - 1

Leaves develop yellow stripes between vascular bundles usually after seedling stage.

Locus 72 v_2 Virescent seedling - 2

Seedling stage is very light yellow turning green slowly.

CHROMOSOME VI

VI is characterized by the gene Y_1 for yellow endosperm found in a large percentage of corn produced throughout the world. Its recessive allele, y_1 , gives white endosperm.

Locus 0 po Polymitotic

Tassels are partially male sterile. Young microspore cells divide in rapid succession without a division of the Chromosomes. No pollen is shed, but, occasionally, seeds are produced when crossed by normal plants.

Locus 13 Y_1 Yellow endosperm - 1

Endosperm is yellow. An additional gene for yellow is endosperm, Y_2 , is located on Chromosome V. Exact location not known.

Locus 33 pg_{11} Pale green seedling - 11

Seedlings are light green or greenish yellow.

Locus 44 Pl Purple plant color

With other genes for plant color, Pl gives dilute purple— $A_1A_2b Pl$; intense purple— $A_1A_2B Pl$; or brown— $a_1A_2B Pl$.

Locus 45 *Bh* Blotched aleurone

Aleurone is "blotched" in the presence of other color genes *A c R*. *Bh* partially takes the place of *C*.

Locus *su₂* Sugary endosperm - 2

Locus 54 *sm* Salmon silk

With the *P^{rr}* gene for red pericarp, silks have salmon color, brown without pericarp color.

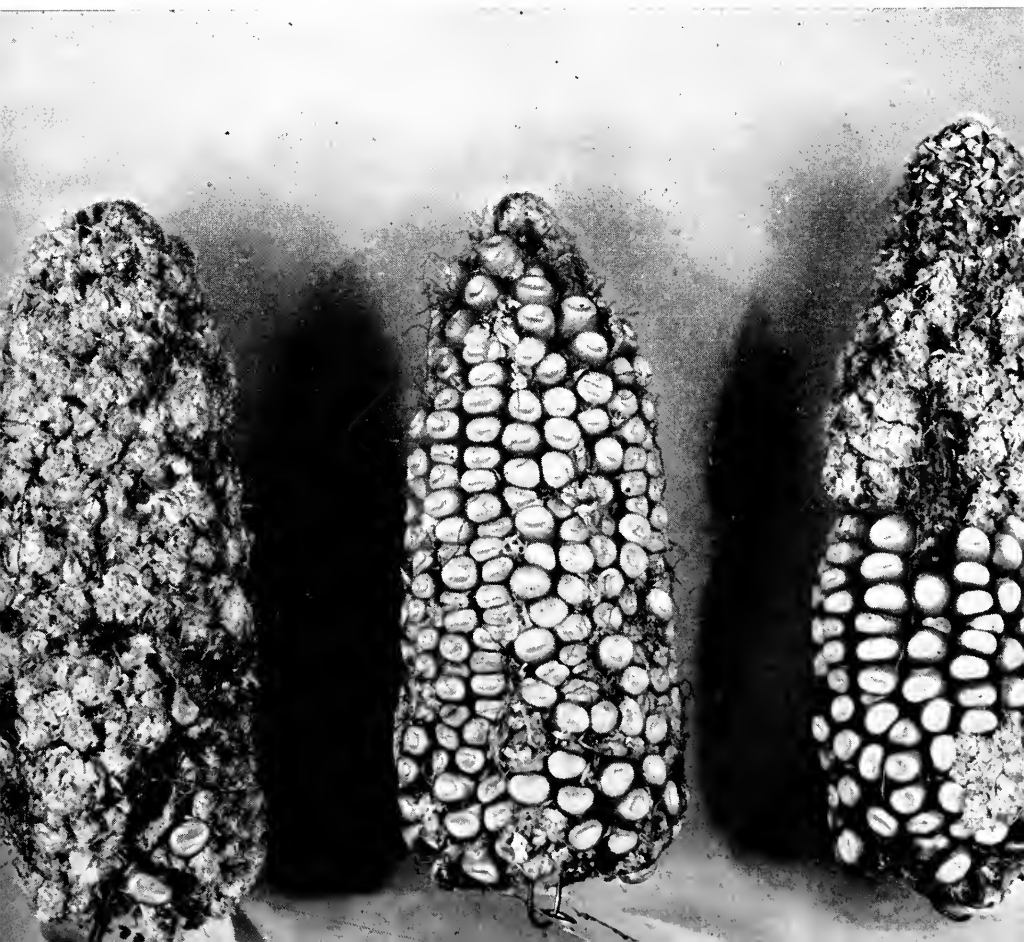
Locus *Pt* Polytypic

Abnormal number of florets produced. Exact location not known.

Locus 64 *py* Pigmy

Plants are short with short, thick leaves which have longitudinal striations.

Pt Polytypic





Hs Hairy sheath

CHROMOSOME VII

This chromosome also has a gene for fertility restoration linked with *gl*₁. (11)

Locus 0 *Hs* Hairy sheath

Leaf sheaths hairy throughout development.

Locus 16 *o*₂ Opaque endosperm - 2

Endosperm is soft with a dull, opaque appearance. It has little or no corneous starch.

Locus 18 *y*_s Lemon yellow endosperm - 8

Locus 20 *in* Intensifier

Intensifies color of purple or red aleurone.

Locus 24 *v*₅ Virescent seedling - 5

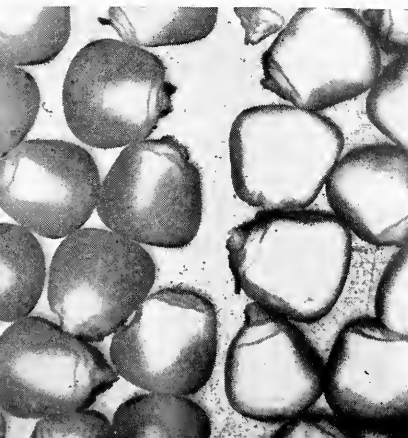
Seedlings are yellow-green turning green quickly. White stripes as in *f*₁ (Chromosome I) may appear later.

Locus 32 *ra*₁ Ramosa ear - 1

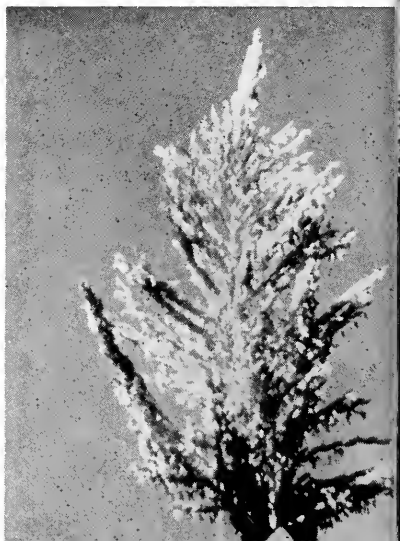
Locus 36 *gl*₁ Glossy seedling - 1

Seedling leaves have glossy appearance. (See *gl*₃, Chromosome IV).

*O*₂ Opaque endosperm



*ra*₁ Ramosa
ear (and
tassel)



Locus 46 *Tp* Teopod

Plants are strongly tillered and have many small, podded ears. Staminate inflorescence has long bracts. Some plants do not shed pollen. They have a greater number of nodes than normal plants.

Locus 50 *sl* Slashed leaf

Leaves split and torn longitudinally. Similar to *Rg*, Chromosome III, but not as severe.

Locus 52 *ij* Iojap striping

Plants vary from albino to variegated. Stripes show throughout life of plant. Expression of this character is poor in presence of *R^{rr}* or *R^{gg}*.

Locus 71 *Bn₁* Brown aleurone - 1

Has pale yellow aleurone color which shows only in the absence of purple and red aleurone. Not to be confused with light yellow or lemon endosperm.

Locus 109 *bd* Branched silkless

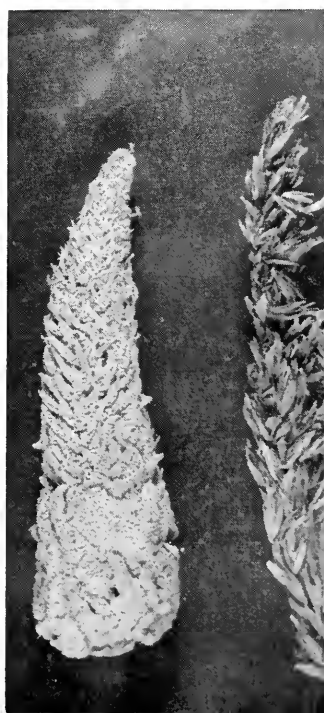
Ears are branched at the base without silks. Spikelets of the tassel occur in groups of more than two.

sl Slashed leaf



Tp Teopod (weak)

bd Branched silkless





Pn Papyruscent glume

Locus 112 *Pn* Papyruscent glume (12)

A dominant mutant characterized by glumes with a thin, papery texture similar to that of ligules on normal leaves. These glumes are usually longer than the mature grain.

Locus _____ *g₂* Golden - 2

CHROMOSOME VIII

Locus 0 *v₁₆* Virescent seedling - 16

Seedlings yellowish green.

Locus 14 *ms_s* Male sterile - 5

One of several genes which cause pollen to abort. Anthers are shriveled and may or may not exert.

Locus 28 *j₁* Japonica - 1

Has a variegated white striping which does not show in the seedling stage. Classification of this gene is good except in the presence of *R* alleles, *R^{rr}* and *R^{gg}*.

Locus ____ *mn* Miniature seed (13)

ms_s Male sterile below
normal above



mn Miniature seed below
normal above





Dt Dotted aleurone

CHROMOSOME IX

This chromosome contains a gene, which in the recessive condition, produces "waxy" starch in the endosperm. Waxy starch contains 100% amylo-pectin useful in the manufacture of certain food stuffs.

Locus 0 *Dt* Dotted aleurone

Gives dots of aleurone color in combination with a_1A_2 *C R*.

Locus 7 *yg₂* Yellow green - 2

Both seedling and older plant yellow-green in color.

Locus 26 *C* Aleurone color

With A_1A_2RPl gives strong purple aleurone color. *I*, inhibitor of aleurone color, is an allele of *C*.

Locus 29 *sh₁* Shrunken endosperm - 1

When mature kernels dry at maturity the endosperm collapses leaving a smooth indentation at the crown. The sides of the kernels may also collapse.

Locus 31 *bz* Bronze

Modifies plant color in the presence of various combinations of *BPl*. It also gives a bronze appearance to red and purple aleurone.

Locus 44 *bp* Brown pericarp

Produces a brown pericarp color with gene *Pl*, Chromosome I.

Locus 59 *wx* Waxy endosperm

Waxy starch, 100% amylo-pectin, reacts to weak iodine solution with a reddish-brown stain instead of the blue-black of normal starch.

Middle—*sh₁* Shrunken endosperm - 1

Bottom—*wx* Waxy endosperm

yg₂ Yellow green - 2 (left)





bk_2 Brittle stalk - 2



pg_{12} Pale green seedling - 12
(at right)

Locus 66 pg_{12} Pale green seedling - 12

Locus 71 v_1 Virescent seedling - 1

Seedlings yellowish green turning green early in their development.

Locus 74 bk_2 Brittle stalk - 2

Stalks and leaves easily broken under pressure.

Locus 106 Wc White cap

Produces white cap on kernels in presence of yellow endosperm.



v_1 Virescent seedling - 1 (at right)

CHROMOSOME X

This chromosome has a gene *Rp* for resistance to the *Puccinia sorghi* rust organism. It also contains the locus *R* which in combination with other genes produces a wide variety of plant colors.

Locus 0 *Rp* Rust resistant

Resistant to Race 3 of *Puccinia sorghi*.

Locus 16 *Og* Old-gold stripe

Light green or yellow striping of chlorophyll begins after fifth or sixth leaf stage.

Locus 24 *nl*₁ Narrow leaf - 1

Plants have narrow leaf blades. They are generally weaker than normal. Leaves tend to be striated longitudinally.

Locus 33 *du* Dull endosperm

When together with *su* it produces super sugary endosperm. With *su*^{am} it gives amy-laceous sugary endosperm.

*nl*₁ Narrow leaf - 1

du Dull endosperm





zn Zebra necrosis



R^{mt} Plant color



w₂ White seedling - 2

Locus 35 *zn* Zebra necrosis

Gives a cross band pattern of necrotic tissue on the leaves.

Locus 38 *l_s* Luteus

Yellow seedling.

Locus 47 *g₁* Golden - 1

Mature plants have a pronounced yellow green color.

Locus 61 *R* Plant color

Produces purple and red colored aleurone and plant in appropriate genotypes. If present as *rrR* in the endosperm it will give mottled aleurone with *Mt*.

Locus 77 *w₂* White seedling - 2

A lethal character. White seedlings live until the food supply from the endosperm is exhausted.

Locus 86 *sr₂* Striate - 2 (14)

Locus 103 *l₂* Luteus - 2

Produces a yellow seedling which is lethal.

Appendix B—Linkage Map of the Mouse*

MARGARET C. GREEN AND MARGARET M. DICKIE†

This map summarizes the most recent information on linkage in the house mouse. Most of it is based on published material but it also makes use of information from personal communications and from Mouse News Letter (a mimeographed bulletin distributed to research workers interested in mice). The unpublished information is used by permission of the authors. (Linkage map is on page 124.)

Mutant genes have been included or excluded from the map on the basis of the following criteria:

1. No genes known to be extinct have been included.
2. The numerous alleles at the histocompatibility loci and at the *t* locus have not been listed.
3. Only linkages published before June 1958 or noted in Mouse News Letter through No. 19, July 1958, have been included. Further information on some of these linkages has been supplied by personal communication.

The distances between loci shown are recombination percentages. They are of varying degrees of reliability, that for the distance between *c* and *p* in linkage group I, for instance, being based on tests with about 24,000 mice, while for some others the number of mice classified is less than 100. The linear order of many of the genes is subject to some degree of uncertainty. Those indicated by symbols in italics have not been critically tested to determine their position relative to all other genes in the linkage group. The order of the other genes is established with a fairly high probability, but it would not be too surprising if further evidence showed some of these to be incorrectly placed. In particular, *ru* and *je* in linkage group XII show only very loose linkage or, in some bodies of data, none at all. They may therefore be in different linkage groups.

The names of the genes of the mouse are given in the following list. The date following the number of each linkage group is the year the first linkage in that group was discovered.

I 1915

fr
ol
sh-1
c-series
c^{ch}

frizzy
oliogodactyly
shaker-1

chinchilla

* Green, Margaret C., and Margaret M. Dickie (1959). Linkage map of the mouse *J. Heredity* 50: 2-5.

† Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

<i>c^h</i>	Himalayan
<i>c^e</i>	extreme dilution
<i>c</i>	albino
<i>hf</i>	hepatic fusion
<i>H-1</i>	Histocompatibility-1
<i>p</i>	pink-eyed dilution
<i>tp</i>	taupe
<i>qv</i>	quivering
<i>da</i>	dark
<i>pu</i>	pudgy
<i>Hk</i>	Hook
II 1927	
<i>se</i>	short ear
<i>d</i> series	
<i>d</i>	dilute
<i>d^l</i>	dilute-lethal
<i>lu</i>	luxoid
<i>du</i>	ducky
III 1931	
<i>s</i>	piebald
<i>ag</i>	agitans
<i>hr</i> series	
<i>hr</i>	hairless
<i>hr^{rh}</i>	rhino
<i>wl</i>	wabblers-lethal
<i>pi</i>	pirouette
<i>W</i> series	
<i>W</i>	Dominant spotting
<i>W^v</i>	Viable dominant spotting
<i>W^j</i>	Jay's dominant spotting
<i>W^a</i>	Ames dominant spotting
<i>Ph</i>	Patch
<i>lx</i>	luxate
<i>rl</i>	reeler
IV 1930	
<i>r</i>	rodless retina
<i>si</i>	silver
<i>pg</i>	pygmy
V 1935	
<i>Ra</i>	Ragged
<i>mg</i>	mahogany
<i>H-3</i>	Histocompatibility-3
<i>kr</i>	kreisler
<i>bp</i>	brachypodism
<i>a</i> series	
<i>A^y</i>	Yellow
<i>A^w</i>	White-bellied agouti
<i>a^t</i>	black and tan
<i>a</i>	non-agouti

<i>a^e</i>	extreme non-agouti
<i>un</i>	undulated
<i>we</i>	wellhaarig
<i>pa</i>	pallid
<i>ro</i>	rough
<i>fi</i>	fidget
<i>Sd</i>	Danforth's short tail

VI 1939

<i>N</i>	Naked
<i>Ca</i>	Caracul
<i>Ht</i>	Hightail
<i>hl</i>	hair-loss
<i>bt</i>	belted

VII 1939

<i>Re</i>	Rex
<i>Al</i>	Alopecia
<i>ti</i>	tipsy
<i>Tr</i>	Trembler
<i>sh-2</i>	shaker-2
<i>vt</i>	vestigial tail
<i>wa-2</i>	waved-2

VIII 1942

<i>m</i>	misty
<i>Pt</i>	Pintail
<i>wi</i>	whirler
<i>b</i> series	
<i>b^c</i>	cordovan
<i>b</i>	brown
<i>Lt</i>	Light
<i>an</i>	anemia
<i>vc</i>	vacillans

IX 1942

<i>T</i> series	
<i>T</i>	Brachyury
<i>t</i>	tailless
<i>Fu</i> series	
<i>Fu</i>	Fused
<i>Fu^{ki}</i>	Kinky
<i>tf</i>	tufted
<i>H-2</i>	Histocompatibility-2

X 1945

<i>v</i>	waltzer
<i>ji</i>	jittery

XI 1948

<i>ob</i>	obese
<i>mi</i> series	
<i>M^{iwh}</i>	White

<i>mi</i>	microphthalmia
<i>px</i>	postaxial hemimelia
<i>wa-l</i>	waved-l
<i>Lc</i>	Lurcher
	XII 1948
<i>ru</i>	ruby eye
<i>je</i>	jerk
	XIII 1953
<i>Lp</i>	Loop tail
<i>py</i>	polydactyly
<i>Dh</i>	Dominant hemimelia
<i>ln</i>	leaden
<i>Sp</i>	Spotch
<i>fz</i>	fuzzy
	XIV 1953
<i>cr</i>	crinkled
<i>ch</i>	congenital hydrocephalus
<i>f</i>	flexed tail
	XV 1956
<i>Tw</i>	Twirler
<i>ax</i>	ataxia
	XVI 1957
<i>Va</i>	Varitint-waddler
<i>de</i>	droopy-ear
	XVII 1958
<i>sa</i>	satin
<i>bg</i>	beige
	XX 1952
	Sex-linked
<i>Bn</i>	Bent
<i>Ta</i>	Tabby
<i>Dp</i>	Dappled
<i>Mo</i> series	
<i>Mo</i>	Mottled
<i>Mo^{br}</i>	Brindled
<i>To</i>	Tortoise
<i>jp</i>	jimpy

Glossary

Acentric. Lacking a centromere.

Acquired character. A change from the normal type brought about by environmental influence.

Adenine. A nitrogen base, one of two purines found in both DNA and RNA.

Adipose mice, ad/ad. Mice with a genetic type of obesity, conditioned by a single recessive gene difference from normal.

Agglutinin. A specific antibody in blood plasma or serum, causing agglutination (clumping) of blood cells with an incompatible agglutinin. Agglutinins may occur naturally, or may develop as a response to the introduction of an agglutino-gen (*see* Antibody).

Agglutininogen. A specific antigen, present normally on some red blood cells, that reacts with a specific agglutinin to cause clumping or agglutination. *A.* may stimulate agglutinin production when injected into an animal (*see* Antigen).

Agouti. The gray-brown coat color of certain wild animals. Individual hairs are black with a subapical band of yellow near tip. The designation comes from an animal species of the same name.

Albescent. A seedling type in maize. Seedlings emerge green and later become whitish, with variable expression.

Albinism. Absence of melanin pigment in animals; absence of chlorophyll in plants. *A.* is usually conditioned by a recessive gene (*c/c* in animals, *w/w* in plants).

Albino seedling. A white seedling due to a recessive gene.

Albino squirrel population. A large population (*ca.* 700) of albino squirrels descended from a single pair of albinos released some 60 years ago in Olney, Illinois.

Aleurone. Protein matter in the form of grains occurring in the endosperm of ripe seeds.

Aleurone layer. The external layer of cells in the endosperm of cereals. Aleurone grains are located in this single layer of cells.

Alkapton. Homogentisic acid, a substance excreted in the urine of persons who are recessive homozygotes lacking an enzyme necessary for the degradation of alkapton into final products CO_2 and H_2O .

Alkaptonuria. A disease caused by a single recessive gene difference from the normal. Homozygous recessives (*a/a*) excrete excessive amounts of alkapton, a strong reducing agent that turns black upon exposure to air.

Alkaptonuriac. A homozygous recessive (*a/a*) person with alkaptonuria.

Allele. One of two normally alternate forms of a gene, one being the normal wild type (+), the other the mutant type. When three or more alleles exist for any given locus (site) in the chromosome, they form a multiple allelic series. Such cases are less frequent than those with only two alleles at a locus.

- Allopolyploid.** A polyploid organism derived from three or more chromosome sets of different species.
- Amphidiploid.** A species or type derived from doubling the number of chromosomes found in the original F_1 hybrid of two different species; an allopolyploid.
- Anaphase.** A stage in cell division following metaphase in which the daughter chromosomes, pass from the equatorial plate in metaphase midway to the two poles at the opposite ends of the cell. The *a.* is actually part of a continuous process of cell division, interrupted in its progress by being killed and stained.
- Aneuploid.** An organism or cell containing a chromosomal complement different from the normal n for gametes and $2n$ for somatic cells; e.g., $2n + 1$, or $2n - 1$.
- Anther.** In seed plants the part of the stamen where pollen grains (microspores) are formed.
- Anthocyanin.** A soluble glucoside pigment producing either reddish or purplish color in flowers and other parts of plants.
- Antibiotic.** A substance usually derived from fungi, capable of destroying life, especially of bacteria.
- Antibody.** A substance (usually a protein) in tissue or fluid (as blood plasma or serum of an animal) that acts in antagonism to specific foreign bodies. Antibodies may exist normally; also the introduction of foreign bodies or substances (antigens) results in the development of specific antibodies (*see* Agglutinin).
- Antigen.** A substance (usually a protein), capable of stimulating antibody production when introduced into an animal (*see* Agglutination).
- Antimutagen.** A substance partially counteracting the effect of a mutagen; e.g., anoxia tends to counteract the mutagenic effects of ionizing radiation.
- Apomixis.** Reproduction in which fertilization does not occur, so that resulting seed represents a vegetative propagation, usually of an unreduced female gamete.
- Ascospore.** One of the spores contained in the ascus of certain fungi (*Neurospora* is a good example). Each meiosis produces eight ascospores, all in a single ascus, or sac.
- Ascus.** In ascomycetes, the membranous tubular spore sac containing eight haploid ascospores.
- Asexual reproduction.** Any method of reproduction not involving the formation of sexual cells or gametes. Examples are budding, grafting, bulbs, tubers in plants.
- Asynapsis.** Failure of chiasma formation during meiotic prophase. A high frequency of univalents results.
- Autopolyploid.** A polyploid species with more than two sets of chromosomes (genomes) all derived from the same original species.
- Autosomes.** Chromosomes other than sex chromosomes. Chromosomes 2 and 3 in *Drosophila* are termed autosomes when balance in sex determination is considered.
- Autotetraploid.** A plant with four genomes, derived by doubling the two genomes of a single diploid species.
- Auxotroph.** A mutant in such lower forms as bacteria and *Neurospora*, requiring some growth factor in addition to a minimal medium.
- Backcross.** A cross of an F_1 hybrid to either of its parents.
- Bacteriophage.** *See* Phage.
- Balanced lethal.** A lethal system in which one genetic line has one lethal, and a second line a different one. Homozygous lethal zygotes are nonviable. Each of two lines possesses a normal allele of the lethal allele of the other. Consequently, the F_1 hybrid is viable, whereas homozygous lethal parental types die, maintaining permanent hybridity with concomitant heterosis.
- Balance in *Drosophila* sex determination.** Sex in *Drosophila* is determined by ra-

- ratio of X chromosomes to autosomes (chromosomes 2 and 3) X/A ratio of 1.0 is female, .5 is male, while .67 and .75 are intersexes.
- Basic number.** The haploid number of the diploid species of a polyploid series. All chromosome numbers in a polyploid series are divisible by the basic number.
- Biochemical basis of heterosis.** A hybrid between two types, each deficient in the ability to synthesize some chemical, grows normally since each type has a normal allele for the deficiency of the other. Consequently the F_1 hybrid has much hybrid vigor, or heterosis, in comparison with the parents.
- Biochemical genetics.** The branch of genetics concerned with the inheritance of genetic differences in the ability or inability to synthesize or metabolize certain chemicals.
- Biotype.** Pure line; a term coined by George H. Shull to describe pure lines produced in corn by inbreeding.
- Bivalent chromosomes.** Two homologous chromosomes paired at meiotic metaphase.
- Calico cat.** A female cat with black and yellow markings. This is a manifestation of the hybrid between black and yellow cats. The gene for black vs. yellow is sex-linked (in X chromosome). A male with one X chromosome is either black or yellow, never calico.
- Carcinogen.** Any cancer-inducing substance.
- Carrier.** An individual heterozygous for a recessive gene that is not expressed. In appearance it is indistinguishable from the homozygous dominant.
- Centromere.** The spindle fiber attachment region of a chromosome. Usually the *c.* is cytologically distinguishable from the rest of the chromosome.
- Character.** A trait, quality, property, or function distinguishing an individual. A mutant character may be conditioned by a single gene difference from the normal; or several genes may interact to produce a given character.
- Chemical mutagen.** One of many chemicals that is capable of inducing mutations.
- Chiasma (pl. Chiasmata).** The point of interchange between chromatids of homologous chromosomes at meiotic prophase; thought to be the place where crossing-over occurs.
- Chimera.** An association of tissues of different genetic origin and constitution in the same part of an organism.
- Chi-square (χ^2).** A statistical test to determine whether observed ratios differ significantly from an expected ratio. $\chi^2 = (d^2/e)$, where d = deviation and e the expected ratio.
- Chromatid.** A daughter chromosomal strand during prophase and metaphase of meiosis and mitosis, before separation at anaphase. Following separation, chromatids become daughter chromosomes.
- Chromatin.** A deeply staining protoplasmic material consisting of mostly DNA, being the principal constituent of chromosomes.
- Chromocenter.** In salivary gland preparations the small irregular-shaped mass of heterochromatin from the X chromosome and the autosomes, plus all of the Y chromosome which is entirely heterochromatin. The euchromatic arms of the salivary chromosomes radiate from the chromocenter.
- Chromogene.** A heredity determiner in the chromosome, in contrast to determiners in the cytoplasm (plasmagenes). (See Gene.)
- Chromomere.** A small, deeply staining particle in the chromosome, recognizable in prophase of meiosis.
- Chromosome.** A deep-staining, rodlike body in the nucleus of cells, visible at cell division. Chromosomes contain the genes, the hereditary determiners. All species have characteristic chromosome numbers.

Chromosome complement. *See* Genome.

Chromosome mapping. The location by linkage test of genes in the different chromosomes in linear fashion, by the strength of the linkage of the genes.

Cis position—pseudoallele. The location of two closely linked mutant alleles in one chromosome with the two normal alleles in the homologous chromosome.

Cistron. The region in a chromosome in which all mutants show a *cis-trans* position effect, i.e., are noncomplementary.

CLB method. A method for detecting all recessive genes (including lethals) in the X chromosome of *Drosophila*. *See* text, Chapter 17.

Coincidence coefficient. A decimal fraction obtained by dividing actual observed double crossovers by the expected double crossovers based on independent occurrence (*see* Interference).

Colchicine. A poisonous alkaloid, $C_{22}H_{25}NO_6$, derived from the autumn crocus, *Colchicum autumnale*. Used as an agent for interrupting mitosis in plant cells, and in minute concentrations for the treatment and prevention of gout.

Color blindness. Inability to distinguish certain colors. Red-green color blindness is inherited as a sex-linked recessive gene in the X chromosome in human beings.

Color inhibitor. One of a number of dominant genes that preclude color formation.

Complementary genes. Two or more genes, neither of which is capable of producing a phenotypic effect, but which "complement" each other and work as a team to produce an effect; e.g., at least one dominant allele of genes *A*, *C*, and *R* is necessary to produce color in maize aleurone.

Conjugation. Joining or pairing in a lengthwise association of homologous chromosomes in meiosis; the joining of *Paramecia* and other protozoans prior to fertilization.

Continuous variation. Variation that is not separable into discrete classes, but rather encompasses a wide range from one extreme to the other. It is determined by multigenic or polygenic inheritance.

Corn grass. A grassy type of corn conditioned by a single dominant gene difference from normal.

Corolla. The petals of a flower joined collectively into a floral envelope, surrounding the pistil and stamens.

Cotyledon. The first leaf, or one of the first pair or whorl of leaves, in seed plants.

Coupling. (In linkage) the linking of two recessive or two dominant alleles, e.g., in a heterozygote *AB/ab*. *Cis* arrangement of genes. (*See* Repulsion.)

Cremello horse. A homozygote for the dilution gene for coat color, *D/D*. The *c.h.* is cream-colored with blue eyes. The heterozygote, *D/d*, is a Palomino.

Crossing-over. An exchange of segments of homologous chromosomes including linked genes at meiosis. The process is inferred from breeding results.

Crossover unit. The per cent of recombination of linked genes. One per cent of recombination equals one crossover unit in a linkage map.

Cumulative effect. The action of two alleles of a gene giving a more pronounced effect than one in the heterozygous condition. Probably one allele supplies insufficient enzyme for the reaction conditioned by two. The hybrids are distinguishable from parents. The effect is sometimes erroneously referred to as incomplete dominance.

Curie. The quantity of any radioactive material giving 3.7×10^{10} disintegrations per second; named for Mme. Marie Curie, the discoverer of radium.

Curly-Lobe-Plum *Drosophila*. A balanced lethal system in chromosome 2 in *Drosophila*. *See* text, Chapter 12, for explanation.

Cyanide production—clover. High cyanide in white clover is dependent upon two complementary genes, each essential to the production of a specific enzyme, and both necessary for cyanide production.

- Cytoplasm.** The protoplasm of the cell surrounding the nucleus.
- Cytoplasmic inheritance.** Inheritance determined by particles in the cytoplasm. *C.i.* is maternally determined, since the sperm is almost completely devoid of cytoplasm, while the egg contains a small amount.
- Cytoplasmic male sterility.** A type of pollen sterility transmitted through the cytoplasm, maternally inherited.
- Cytosine.** A nitrogen base, one of two pyrimidines found in both DNA and RNA (*see also* Thymine).
- Deficiency (deletion).** A chromosome with a segment missing, either internally (interstitial) or from either end (terminal).
- Degrees of freedom.** A statistical designation indicating the number of variables that must be considered when determining whether results are significant. The number of degrees of freedom is usually one less than the total number of variables in the experiment (*see* Chapter 5).
- Delayed segregation snails.** Segregation occurs in F_3 , rather than in F_2 since the phenotype of the individual is determined by the genotype of the mother.
- Deoxyribonucleic acid (DNA).** A nucleic acid found primarily in chromosomes, and considered to be the primary substance of the gene. DNA consists of phosphoric acid, a sugar known as deoxyribose, two purine bases (adenine and guanine) and two pyrimidine bases (thymine and cytosine).
- Detassel.** To remove tassels from corn plants to accomplish cross-fertilization on a field scale. Special rows (usually one in five) are planted to supply pollen for detasseled rows.
- Deviation.** (In statistics) a variation from an expected number.
- Diakinesis.** A stage in meiotic prophase in which the paired chromosomes are much shortened and thickened. The nucleolus is still visible. *D.* precedes metaphase I.
- Differential sensitivity.** A difference between two organisms in sensitivity to environmental conditions; e.g., one may be killed by a temperature that is optimum for the other.
- Dihybrid.** A hybrid for two different genes.
- Dioecious.** Having two sexes in different organisms. Higher animals are dioecious, and so are some plants, e.g., holly, hemp, spinach.
- Diploid (2n).** Having a chromosome number just twice the haploid gametic number. (n). At meiosis homologous chromosomes form pairs; hence the name, meaning double or twofold (*see* Haploid).
- Diplotene (diplonema).** A stage in late meiotic prophase I in which the paired chromosomes have each become doubled so that each pair has four strands. Chiasmata are visible at this stage. Crossing-over also occurs here.
- Discontinuous variation.** Variation in which discrete classes are easily recognized, e.g., tall vs. dwarf plants, yellow vs. green cotyledons in peas (*see* Continuous variation).
- Disjunction.** Separation of chromosomes between metaphase and anaphase in cell division.
- Disomic inheritance.** Normal inheritance dependent upon two alleles (sometimes more) for a given gene, customarily found in diploid species with no extra chromosomes or chromosome sets (genomes).
- Dizygotic twins.** Fraternal twins, produced by the fertilization of two separate eggs by two different spermatozoa.
- Dominance.** Complete suppression of the expression of one allele by another at the same locus in the chromosome. The suppressed allele is termed recessive.
- Double bar (Drosophila).** A more extreme bar phenotype, manifest as a narrower

- bar with reduced facet number, brought about by the duplication of a segment of the X chromosome at the bar locus.
- Double cross.** Not what is done to the hero by the villain in the movie, but a hybrid between two single crosses of different, pure inbred lines. Most commercial corn hybrids are double crosses.
- Double crossovers.** In linkage tests involving three or more genes such as *ABC/abc*, the occasional formation of gametes *AbC*, and *aBc*, representing crossovers at two places.
- Drift (genetic or random).** The chance establishment of genes out of proportion to the frequency in the original population. *D*. is also called the Sewall Wright effect because of Dr. Wright's contribution to understanding it.
- Dt gene maize.** A "genic mutagen" in chromosome 9 in maize, causing *a* (chromosome 3) to mutate to *A*, yielding dotted kernels or leaves streaked with anthocyanin.
- Duplicate genes.** Two gene pairs that produce the same phenotypic effect; e.g., in Shepherd's Purse either of two dominant alleles T_1 or T_2 produces a triangular pod. The double recessive $t_1/t_1\ t_2/t_2$ pod is ovate. The effect of T_1 is indistinguishable from that of T_2 ; hence they are called duplicate.
- Duplication.** A repeated segment of chromosome. Bar "gene" in *Drosophila* is a short duplication.
- Dyad.** A stage in meiosis following the first division in which there are two cells. These later divide to give four cells each with the haploid chromosome number.
- Egg.** The female germ cell; in animals, an ovum.
- Element (of Mendel).** The particulate hereditary determiner (*see* Gene).
- Embryo.** A young organism in the first stages of development; the immediate product of fertilization.
- Embryo sac.** A large thin-walled cell within the nucellus of the ovule of seed plants, in which the egg and the embryo develop.
- Endomitosis.** A doubling of the chromosomes without a division of the nucleus, giving rise to polyploidy. Chromosome strands separate but the cell does not divide (*see* Polyteny).
- Endosperm.** The nutritive tissue surrounding the embryo in seed plants. Tissue is $3n$ formed by the fertilization of two nuclei of a female gametophyte by one sperm.
- Endosperm mutation (maize).** A change that is detected by a difference in the endosperm of the corn kernel, e.g., starchy to sweet $Su \rightarrow su$.
- Environment—external.** The aggregate of all external conditions and influences affecting the life and development of an organism.
- Environment—internal.** The cellular environment affecting the expression or manifestation of certain genes; e.g., hormonal constitution affects expression of the gene for baldness in men and women.
- Environmental determination of sex.** A phenomenon that occurs in a few organisms in which the sex of a sexually undifferentiated organism is determined by the environment. *See* Bonellia in Chapter 13.
- Enzyme.** A complex organic substance (a specific protein) that accelerates (catalyzes) a specific chemical reaction. Genes produce their effects by the activation or inactivation of specific enzymes.
- Enzyme inactivation at high temperature.** In certain animals, e.g., Himalaya rabbits and Siamese cats, melanin is produced at extremities, tips of ears, tail, nose, and on feet where body temperature is lower. The enzyme necessary for melanin production is inactivated at body temperature.
- Epistatic gene.** A gene which suppresses the action of another gene not at the same

- locus in the chromosome. (Suppression at the same locus is called dominance.)
 The gene suppressed is hypostatic (*see* Hypostatic gene).
- Equatorial plate.** The plane in the center of a cell where, at metaphase, chromosomes are assembled.
- Erythroblastosis fetalis.** Hemolytic anemia of newborn infants. *E.f.* sometimes occurs when the father has antigens not possessed by the mother, who then makes antibodies that destroy the red blood cells of the fetus.
- Euchromatin.** The "good" chromatin, largely made up of genes (*see* Heterochromatin).
- Eugenics.** The science concerned with improvement of the heredity of the human race. The province of *e.* has largely been taken over by the newer science of human genetics.
- Euploid.** Of regular normal chromosome numbers of polyploids. Gametes are haploid (n). An organism is usually diploid with exactly ($2n$), but may be $3n$, $4n$, $5n$, $6n$, etc., with exact multiples of n . (*see* Heteroploid).
- Evolution.** The development of varieties, species, genera, and families, and the relationship of these to each other. Genetics has provided a better understanding of the mechanism of evolution (*see* Chapter 21).
- Expected ratio.** In statistical evaluation of an observed ratio, the ratio by which deviations are calculated to determine whether they are significant (*see* Chi square and Chapter 5).
- Experimental alteration of germ plasm.** The changing of the germ plasm so that the inheritance is altered. *E.a.* can be produced by ionizing radiation, ultraviolet light, heat, chemicals, and such novel methods as transduction and transformation. (A more appropriate term than radiation genetics.)
- Expressivity.** The phenotypic expression of a given gene or the degree to which it is expressed. Genes that always produce a given effect have 100 per cent *e.*
- F₁.** Abbreviation for first filial generation, usually the hybrid between two homozygous types.
- F₂.** Abbreviation for the second filial generation, usually produced by intercrossing two F₁'s or by self-fertilizing an F₁, as is possible in plants. Some plants are naturally self-pollinated, so F₁ plants produce an F₂ generation automatically, e.g., peas used by Mendel and beans by Johannsen in his pure line experiments.
- Factor (obsolete).** The hereditary determiner; the element of Mendel. Gene is a more appropriate term (*see* Gene).
- Female plant.** A pistillate plant in a dioecious species. It produces the female gamete, the egg.
- Female sterility (sorghum).** A type which produces no viable eggs, but ample sperm in the pollen; due to complementary genes (*see* Chapter 13).
- Fertility.** The ability to produce offspring.
- Fertility restorers (maize).** Cytoplasmic male sterile lines will produce viable pollen in certain genotypes. Restorers have genes that restore fertility to a cytoplasmic male sterile line.
- Fertilization.** The union of a male gamete (sperm) with an egg (female gamete) to form an embryo. Triple fertilization results when a sperm unites with two female gametes to form an endosperm in higher plants.
- Fetus.** The prenatal stage of mammals, from embryonic stage to birth; in man, from about the third month to birth.
- Four-strand stage crossing-over.** Crossing-over between chromatids at the diplotene stage in meiotic prophase when four chromatids are present.
- Fragmentation.** The breakage of chromosomes or chromatids into two or more fragments.

- Gamete.** A mature reproductive male or female germ cell, sperm or egg.
- Gametic (tissue or generation).** Having n number of chromosomes (haploid) in contrast to zygotic tissue with $2n$ (diploid). Some microorganisms for the most part are haploid.
- Gametogenesis.** Formation of the gametes; microsporogenesis and megasporogenesis in plants; oögenesis and spermatogenesis in animals.
- Gametophyte.** That part of the plant bearing the gametes (haploid, n tissue).
- Gametophytic differences.** Differences observable in the gametophyte, e.g., waxy vs. starchy pollen in corn and in some other cereals. Most, if not all, of the differences in lower forms (haploid) are gametophytic.
- Gaudens (Oenothera).** A gene complex characterized by genes for green buds, nonpunctuate leaves, white nerves, broad leaves, and red flecks on the rosette leaves (*see* Velans).
- Gaudens-velans hybrid (Oenothera).** *Oenothera lamarckiana* is a hybrid of the *gaudens-velans* complexes.
- Gemmule.** One of the hypothetical supramolecular units assumed in Darwin's theory of pangenesis.
- Gene.** The discrete particulate hereditary determiner located in the chromosome in linear order; the "element" of Mendel and "factor" of early genetic terminology. The g is composed mostly of DNA. It was discovered by Mendel and named by Johannsen.
- Gene flow.** (In population genetics and evolution) the method by which mutant genes become established in populations or eliminated from them.
- Gene frequency.** (In population genetics) the frequency of a gene in a population, expressed as a fraction or a decimal fraction, with p representing the normal allele and q representing the mutant. In controlled matings of homozygous types, $p = q = \frac{1}{2}$ and $p + q = 1$. Also $q = 1 - p$.
- Gene reduplication.** The duplication of genes in the chromosomes at cell division so that each daughter gene and chromosome is an exact replica of its original.
- Gene symbols.** A system for designating genes. Mutant recessive genes are usually characterized by a small letter or letters, e.g., m , mt , while the normal dominant allele is indicated by the capital letter (M , Mt) or a plus sign (+).
- Genetic block.** (In biochemical genetics) a block in a step in the synthesis or segregation of a biochemical brought about by a mutant gene.
- Genetic drift.** *See* Drift.
- Genetic equilibrium.** A state in which the frequency of the alleles of a given gene tends to remain constant. Gene frequencies may vary widely from equal values of $\frac{1}{2}$, but after one generation of random mating, they tend to remain the same (*see* Chapters 20 and 21).
- Genetic fine structure.** The smaller parts into which the gene has been subdivided (*see* Cistron; Muton; Recon).
- Genetic isolation.** Isolation of a variety or species by genetic means, e.g., cross sterility in corn and cross incompatibility of amphidiploid species with parents or other species.
- Genetic mutagen.** A gene that causes other genes to mutate, e.g., the *Ac-Ds* system in corn, or the *Dt* gene in corn.
- Genetics.** The science of heredity of the similarities and differences among organisms.
- Genic explanation of hybrid vigor.** Hybrid vigor is widely explained as the result of a dominance of linked genes. Many dominant genes are involved. Different inbreds contribute different dominant genes, so that the F_1 has more than either parent.
- Genic and plasmagenic interaction (Paramecium).** Interaction between a plasma-

- gene *kappa* and the gene *K* in the chromosome to produce a killer type (see Chapter 15).
- Genome.** A set of chromosomes (*n*) inherited as a unit. In a polyploid series a diploid has two genomes, a tetraploid four genomes, a hexaploid six genomes, etc. Gametes have just half as many genomes as the zygote.
- Genotype.** The genetic constitution or gene makeup of an organism. When dominance is involved the genotype can be determined only by breeding. The contrasting term is phenotype.
- Geographic isolation.** Isolation of biological populations by a geographic barrier such as a mountain range, a canyon, or a river.
- Germ cell.** A mature reproduction cell; a gamete, capable of uniting in fertilization with another gamete to reproduce the organism.
- Germ plasm.** A special kind of protoplasm transmitted unchanged from generation to generation. The chromosomes and genes constitute the *g.p.*, the physical basis of heredity.
- Glomerella.** A plant pathogen, one of the ascomycetes with several mating types that show preferential mating with different types.
- Glossy seedling** (in maize and other cereals). A seedling which lacks pubescence and consequently seems shinier or glossier than the norm. The *g.s.* does not shed water like normal, slightly pubescent seedlings; water stands on it in droplets. Several recessive genes will cause glossy seedlings.
- Gonad.** A gland, such as an ovary or testis, in which gametes are produced.
- Gout.** A metabolic disease marked by an excess of uric acid in the blood, accompanied by painful inflammation of the joints. *G.* is caused by a genetic deficiency, most likely an autosomal dominant gene.
- Guanine.** A nitrogen base, one of two purines found in both DNA and RNA (see Adenine).
- Gynandromorph.** An animal in which one part is phenotypically male, the other female.
- Haploid.** Having half the number of chromosomes (gametic number) found in diploid organisms.
- Hardy-Weinberg law.** A law concerning gene frequencies in populations. It states that after one generation of random mating, gene frequencies remain constant in future generations.
- Hemizygous.** Having but one gene (or chromosome) present, with no homologous allele. Genes in the X chromosome are hemizygous, as are genes in haploid organisms.
- Hemophilia.** A tendency to profuse bleeding even from slight wounds. Inheritance is governed by a recessive gene in the nonhomologous portion of the X chromosome in man. *H.* is a sex-linked abnormality.
- Heredity.** Transmission of traits from parent to offspring.
- Heritability.** The extent to which a given trait is determined by inheritance.
- Hermaphrodite.** An individual having both male and female reproductive organs (see Monoecious).
- Heterocaryon.** A binucleate mycelium of microorganisms, e.g., *Neurospora*, with two nuclei of different genotypes.
- Heterocaryotic vigor of fungi.** The hybrid vigor brought about by two different nuclei in a heterocaryon in a single cell of a mycelium.
- Heterochromatin.** The chromatin staining less and having a different genetic function than the euchromatin which contains most of the genes and stains deeply. The Y chromosome of the XY pair is mostly *h.*, with few genes.
- Heterogametic sex.** The sex which has morphologically different sex chromosomes

(XY or XO) and hence produces two kinds of gametes. In *Drosophila* and man the male is the *h.s.* (*see* Homogametic sex).

Heteroploid. Having a chromosome number differing from the normal number ($2n$ for diploids, n for gametic number). Examples of heteroploids are $n + 1$ gametes, or $2n + 1$ or $2n - 1$ zygotes.

Heterosis. Hybrid vigor; a term coined by George H. Shull.

Heterozygote. An individual having different alleles for any gene pair and producing two kinds of gametes.

Heterozygous. Hybrid for any gene pair, with different alleles for the gene considered.

Hexaploid. (Usually) a diploid with duplicate sets of three different genomes. Common bread wheats (42 chromosomes) are hexaploids in contrast to primitive wheat with 14 chromosomes (a diploid).

Homogametic sex. The sex with both sex chromosomes alike and capable of producing but one type of gamete, e.g., female *Drosophila* XX and male poultry XX.

Homogentisic acid. A reducing substance excreted in the urine of persons with a genetic metabolic deficiency known as alkaptonuria. Upon oxidation, *h.a.* turns black. Also called alkapton.

Homologous chromosomes. Chromosomes which pair at meiotic prophase and are similar in size, shape, structure, and function. They have alleles of the same genes.

Homozygote. An individual with both alleles alike for any given gene. Homozygotes are pure for any genetic trait, in contrast to heterozygotes, which are hybrid.

Homozygous. Pure, or having but one type of allele in both chromosomes. A homozygous individual produces but one kind of gamete.

Hornal difference in horn production in sheep. In some sheep hybrids between horned and hornless breeds, the heterozygous male has horns, while the heterozygous females are hornless, presumably an influence of the male and female sex hormones.

Hybrid. A cross of unlike organisms; *adj.*, characterized by such a cross.

Hybrid corn. A cross of two different kinds of corn. The term implies a hybrid of two inbred lines or two F_1 hybrids (double cross). Practically all corn grown in the United States is hybrid.

Hybridization. The process of making a hybrid, by cross-pollination in plants, or by mating two types of animals.

Hybrid vigor. The unusual robustness, rapid growth, and thriftiness of organisms produced by crossing two less vigorous parents; heterosis.

Hypostatic gene. A gene whose phenotypic effects are suppressed by another gene not at the same locus in the chromosome (*see* Epistatic).

Identical twins. Twins derived from one egg, which following fertilization divides to produce two zygotes, both of them having identical genotypes.

Inborn error of metabolism. Metabolic deficiencies inherited in Mendelian manner and produced by recessive genes; a term coined by Sir A. E. Garrod to apply to biochemical mutants in man.

Inbred. (Of maize) characterized by pure lines that have been made nearly homozygous by self-pollination or inbreeding (*see* Biotype).

Inbreeding. Mating of related types; consanguineous matings. In plants self-pollination is the closest possible inbreeding and results in pure lines rather quickly. Next closest inbreeding is mating of sisters with brothers (sib matings).

Incomplete dominance. *See* Cumulative effect.

Independent assortment. Random assortment in which segregation for one type

- has no effect on any other. Alleles in different chromosomes assort independently at gametogenesis.
- Induced chromosome break.** Chromosome break caused by some agent usually external to the chromosome, such as radiation or chemicals. *I.c.b.* can be produced genetically, e.g., the *Ac-Ds* system in maize.
- Inhibitor of aleurone color.** The *CI* gene in maize inhibits color in the aleurone even though *A*, *C*, and *R* are present.
- Interference.** The crossing-over of two linked genes at meiosis, which "interferes" with or reduces crossing-over in regions adjacent to the two linked genes. *I.* is detected when studying crossing-over of three or more linked genes (*see* Coincidence coefficient).
- Interphase.** That stage in cell division following telophase and preceding the next prophase; a more appropriate term than "resting stage."
- Intersex.** A type that shows some phenotypic characteristics of both males and females. Intersexes are not to be confused with gynandromorphs.
- Inversion.** A rearrangement of a chromosome in which a portion is rotated a full 180 degrees. It sometimes results when a chromosome is broken at two places.
- Iojap maize.** A maize plant with green and white stripes, an unusual collaboration of genes and cytoplasm.
- Ionizing radiation.** Any of the high-energy radiations that displace electrons from neutral atoms. *I.r.* destroys the balance between the negative charge of the electrons and the positive charge of the atomic nucleus, making ions out of atoms.
- Isolating mechanism.** (In population genetics and evolution) a way of isolating populations so that the gene frequencies are maintained. *I.m.*'s may be geographic or genetic.
- Isolating mechanism, chromosomal.** A difference in chromosome number or morphology that maintains genetic isolation or lack of crossing.
- Kappa (Paramecium).** A particle in the cytoplasm of *Paramecium* that grows in the presence of gene *K*, producing a killer type.
- Karyolymph.** A clear fluid or nuclear sap within the nuclear membrane.
- Karyotype.** The character of the chromosomal complement with reference to the comparative size, shape, and morphology of the different chromosomes.
- Kinetochore.** *See* Centromere.
- Klinefelter's syndrome (man).** An abnormal type in which the constitution for the sex chromosome is XXY. In such an individual the testes are small and contain no mature sperm.
- Kynurenineless ephestia.** A mutant type in the flour moth *Ephestia* incapable of producing a hormonelike substance (kynurenin) necessary for full pigment production.
- Leptotene (Leptonema).** An early stage in meiotic prophase in which individual chromosomes are first visible as long threads; hence the name.
- Lethal mutation (gene).** A gene which renders a gamete nonviable (gametic lethal) or one which prevents the complete development of a zygote (zygotic lethal). Zygotic lethals may produce their effects at any time from the fertilized egg on.
- Liguleless seedling.** A seedling in maize without a ligule, one of the distinctive features of the family Gramineae. Several recessive genes cause liguleless seedlings.
- Linkage.** The association of two or more characters in inheritance caused by two

genes associated in the same chromosome. The nearer the genes are to each other, the closer the linkage.

Linkage group. A group of characters all showing linkage with others in the same group. The genes for these characters are arranged linearly in one chromosome, the genes close together showing close linkage.

Linkage map—*Drosophila*. A map of the four chromosomes of *Drosophila* showing location of many genes. Map distances are expressed as per cents of crossing-over between genes (see Fig. 8-9).

Linkage map—maize. A map of the 10 chromosomes of maize showing linkage value (crossover per cent) for many genes (see Fig. 8-10).

Linkage map—mice. A map of the 20 mouse chromosomes showing linkage values (see Fig. 8-11).

Linkage value. A recombination per cent expressing the proportion of crossovers to non-crossovers (the parental type). The l.v. may vary from zero to 50 per cent, which is the value for independent assortment.

Locus (pl. loci). The physical location of a gene in the chromosome. The genes have normally two—in some instances more—alleles at the same locus.

Lysis. Destruction of a bacterium, as by bacteriophage, with the multiplication of phage particles in the process.

Lysogenic. Of bacteria which carry temperate phages. The temperate phages do not kill the bacterium, but take up a symbiotic relationship; the bacteria may benefit.

Lysogenic bacteria. Those harboring temperate phages which may cause the lysis of another bacterium.

Male plant. A staminate plant in a dioecious species.

Male sterile maize (cytoplasmic). Corn that produces no viable pollen because of something in the cytoplasm rendering it sterile.

Male sterile maize (genic). A plant producing no viable pollen because homozygous for a recessive pollen lethal usually designated *ms/ms*.

Maternal chromosomes. In bisexual organisms the chromosomes derived from the mother (see Parental chromosomes).

Maternal influence (Ephestia). The influence of the genotype of a heterozygous mother on the eggs and larvae. The dominant color of the mother persists for a short time in eggs and larvae, even with recessive genotype.

Maternal inheritance (cytoplasmic inheritance). Transmission of hereditary determiners in cytoplasm through the maternal side only (the egg contains some cytoplasm, the sperm none); e.g., plastid inheritance in plants, and cytoplasmic male sterility.

Megaspore. The "large" spore that gives rise to the female gametophyte (embryo sac) in which the egg (female gamete) is produced.

Megaspore mother cell (megasporocyte). A diploid cell ($2n$) that undergoes two divisions, one a reductional division, to produce four megaspores.

Megasporogenesis. The process of the production of megaspores from a megasporocyte.

Meiosis. A special type of cell division found in gamete production. It consists of two divisions, one of which is reductional. Homologous chromosomes pair and assort at random to produce gametes with the haploid (n) number of chromosomes.

Melanin. A black or brown pigment of animal origin. In albinos, a mutant recessive gene blocks the production of melanin.

Mendelian population. A naturally breeding unit, isolated by some mechanism from other units, of sexually reproducing plants or animals.

- Mendel's law.** The principle that hereditary characters are determined by discrete particles (genes) that segregate at random in gamete formation (*see also* Linkage).
- Metacentric.** Of a chromosome (or chromatid) having the centromere at or near the middle, lengthwise.
- Metaphase.** The stage in cell division at which the chromosomes are shortened and arranged on an equatorial plate in the center of the cell.
- Microspore.** The "small spore," an asexual spore, the pollen grain in plants, in which develop the male gametes, the sperm.
- Microspore mother cell.** A diploid cell that goes through two divisions, one a reductional, producing four microspores. It is the same as the pollen mother cell.
- Microsporogenesis.** The process in which microspores are produced. Meiosis produces four cells that, when mature, are the microspores (pollen grains).
- Microsurgery by radiation.** The breaking of a chromosome in two places and the insertion of a fragment into another chromosome that has likewise been broken.
- Mid-prophase.** The stage following zygotene when paired chromosomes are somewhat shortened and thickened, a stage at which chromosomes have been studied extensively. Also called pachytene.
- Milk factor in cancer transmission.** A factor present in the milk of mice that transmits cancer.
- Minimal medium for Neurospora.** A medium containing certain inorganic salts, a suitable carbohydrate, such as sucrose, and the vitamin Biotin.
- Mitosis.** Cell division in which chromosomes are all divided equationally so that daughter cells have exactly the same number and composition as the original.
- Modifying gene.** A gene which alters the expression of another gene that is non-allelic, e.g., genes affecting the amount of spotting in Holstein cattle, a spotted breed.
- Monococious.** Having male and female reproductive organs in the same individual. In plants, staminate and pistillate flowers occur in the same plant, e.g., corn.
- Monogenic.** Having inheritance determined by a single gene difference from wild.
- Monohybrid.** *See* Monogenic.
- Monoploid.** An individual having but a single genome or set of chromosomes (*see* Haploid). Haploids of diploid species are monoploids. However, haploids of polyploids are not monoploids, since they contain more than one genome.
- Monosomic.** Having a full set of chromosomes minus one chromosome, $2n - 1$. (*See* Nullisomic; Trisomic.)
- Monozygotic twins.** Twins that have developed from a single fertilized egg that divided to form two individuals; identical twins.
- Mulatto.** The F_1 offspring of a pure Negro and a pure white.
- Multigenic inheritance.** Inheritance determined by several genes with cumulative effect, e.g., ear length in corn.
- Multiple alleles.** More than the normal two alleles at a locus in the chromosome.
- Mutable gene.** An unstable gene that mutates frequently.
- Mutagen.** Any substance, chemical or physical, that causes genes to mutate. Some genes are mutagenic.
- Mutant.** A variant from the normal or wild type that is inherited in a Mendelian manner.
- Mutation.** A change that is inherited (*see* Mutant).
- Mutation pressure.** A constant mutation rate that adds mutant genes to a population.
- Mutator gene.** A gene that causes other genes to mutate; e.g., *Dt* in corn causes *a* to change to *A*.
- Muton.** A subdivision of the gene; the smallest element, alteration of which can be effective in causing a mutation.

- Mycelium** (pl. **Mycelia**). One of the mass of interwoven threadlike filaments forming the vegetative portion of the thallus in fungi.
- n, 2n**. The genetic (haploid) and zygotic (diploid) chromosome numbers respectively
- Natural selection**. Survival of the fittest. "Fitness" is measured by the ability to leave progeny.
- Nondisjunction**. The failure of two homologous chromosomes to separate or disjoin at meiosis, so that one daughter chromosome receives two, the other none of a given chromosome pair.
- Nonrandom assortment**. Assortment or segregation of genes by a process that is not random; e.g., linked genes show nonrandom assortment.
- Nonrandom mating**. Selective mating when specific phenotypes show a preference for a particular phenotype; e.g., Negroes tend to marry Negroes and whites to marry whites.
- Nullisomic**. The complete lack of a given chromosome pair; $2n - 2$.
- Octoploid**. An individual with eight genomes or monoploid sets of chromosomes. Octoploids customarily form chromosome pairs at meiosis and not n sets of eight.
- Oöcyte (primary)**, **2n**. The egg mother cell giving rise to the egg and a polar body at the first division, both with n chromosomes.
- Oöcyte (secondary)**, **n**. The haploid cell following the first meiotic division that divides equationally to produce the egg (female gamete) and an additional polar body which disintegrates.
- Oögenesis**. The formation of the egg in animals.
- Oögonium**. A diploid primary germ cell in the female animal, usually found in the early embryo. The *o*. gives rise to primary oöcytes, secondary oöcytes, and eggs (the female gametes).
- Origin of species**. The production of new types of organisms in descent from extant types. Natural selection is the process by which changes are favored leading to the production of new types. The term was introduced by Charles Darwin.
- Outbreeding**. Mating of unrelated individuals or of individuals not closely related; the opposite of inbreeding.
- Ovary**. The female reproductive organ in which eggs are produced. In plants, the ovary, containing ovules, is at the base of the pistil.
- Overdominance**. An effect in the heterozygote, A/a , greater than in the homozygous dominant, A/A .
- Ovule**. The megasporangium of a seed plant. After fertilization it becomes the seed.
- P**. Abbreviation for parent.
- Pachytene (pachynema)**. Mid-prophase in which the chromosomes are visible as long, paired threads.
- Palomino**. A popular breed of horses with golden color and white mane and tail. The color is produced in a heterozygote, or hybrid, for the dilution gene D/d , which shows a cumulative effect with no dominance.
- Pangensis**. A discarded theory (Darwin) proposed to explain heredity. Somatic cells were supposed to throw off minute granules (gemmules) that accumulated in reproductive organs.
- Paracentric inversion**. A rotation of a segment of chromosome a full 180° , with the centromere beyond inversion, which is all within one arm of chromosome. *Para* means "alongside of," "beyond."
- Paramutation**. A mutation in which one allele in heterozygous condition changes permanently its partner allele (see Chapter 22).

Parthenogenesis. Reproduction without fertilization.

Paternal. Derived from the father.

Paternal chromosomes. Chromosomes derived from the father.

Pedigree. (1) The genotype of an individual; (2) a chart showing the ancestral history of an individual.

Penetrance. The extent to which a gene produces a phenotypic effect. Recessive genes have no penetrance in the heterozygote. Some dominant genes always produce a given phenotype (complete penetrance). Others may fail under certain conditions to produce a given phenotype (incomplete penetrance). (See Expressivity.)

Pericarp. The outer layers of cells surrounding the seed (see Fig. 9-1).

Pericentric inversion. A chromosomal inversion of 180° in which the inverted segment includes (surrounds) the centromere.

Periclinal chimera. One tissue completely surrounding another type of tissue.

Permanent heterosis in *Oenothera*. A hybrid condition maintained by a balanced lethal system so that *Oenothera lamarckiana* is a permanent hybrid of *gaudens-vulans* complex.

Phage (bacteriophage). A virus which may kill bacteria (virulent) or may invade bacteria and exist in a symbiotic relationship (temperate). Genetic recombination occurs in both temperate and virulent phage.

Phenocopy. A nonheritable, environmentally induced modification resembling the phenotype of a known mutant gene.

Phenotype. The appearance of an individual produced by the genotype in cooperation with the environment.

Phenylalanine. An amino acid which serves as a building block for proteins. Several genetic blocks occur in phenylalanine metabolism, giving rise to such conditions as albinism, alkaptonuria, phenylketonuria, etc.

Phenylketonuria. A disease inflicting serious brain damage in infants, caused by a recessive gene. It renders a child unable to metabolize phenylpyruvic acid, which accumulates in the brain.

Phenylpyruvic idiot. The abnormal individual produced by a particular recessive gene (see Phenylketonuria).

Photoperiodism. The action of certain amounts of light stimulating floral initiation in plants.

Piebald spotting. (Usually two) colors on an animal in rather large blotches of color.

Pinto horses. Piebald-spotted horses, caused by a dominant gene.

Pistil. The ovule-bearing organ of a flower, at the base of the style.

Pistillate. Bearing female gametes only; of a female type with no stamens.

Plasmagene. A unit in the cytoplasm causing hereditary traits, e.g., *kappa* in *Paramecium*.

Plastids. Small bodies of specialized protoplasm, especially in plant cells, e.g., chloroplasts containing the green pigment chlorophyll.

Pleiotropic. With multiple effects (said of a given gene), usually in parts of the organism not obviously related—e.g., flower color and seed coat color.

Polar body. One of the minute cells which separate from the animal egg during its maturation. The *p.b.* does not take part in fertilization. Each primary oöcyte produces one egg and three polar bodies.

Pollen grain. The microspore in plants containing a tube nucleus, and a generative nucleus that divides either in pollen grain or in pollen tube to form two sperm nuclei.

Pollen mother cell. The microsporocyte ($2n$) in plants immediately before the reduction division. It is the place where meiotic chromosomes are readily studied.

Pollen parent (male parent). The plant supplying the pollen for a hybrid.

- Polygene.** One of many genes necessary for a given phenotypic effect as found in quantitative inheritance.
- Polyploid.** Composed of more than two genomes, or chromosome sets. $2n$ = diploid, $3n$ = triploid, $4n$ = tetraploid, etc.
- Polyteny.** Division of the chromosomes without a division of the nucleus, resulting in multistranded chromosomes, as in salivary gland chromosomes of *Drosophila* and some other Diptera (*see* Endomitosis).
- Population genetics.** The branch of genetics concerned with the frequencies of genes (alleles) in a population.
- Position effect.** A definite phenotypic expression depending on the arrangement of genes in relation to neighboring genes.
- Prejvalski horse.** The wild type ancestor of the domestic horse.
- Probability.** The chance or likelihood of a given possible event.
- Progeny.** Offspring; individuals resulting from a mating.
- Prophase.** A stage of cell division in which chromosomes are first visible as chromosomes. The *p.* precedes metaphase.
- Prototroph.** A "wild type" bacterium that will grow on a minimal medium.
- Pseudoalleles.** "False" alleles, once thought to be alleles, but which have been shown to be separable by crossing-over.
- Pseudoalleles cis arrangement.** Arrangement of two mutant alleles in one chromosome strand, with the wild type alleles in the other.
- Pseudoalleles trans arrangement.** A mutant allele and a wild type allele in each of two chromosome strands.
- PTC (phenyl-thio-carbamide).** A substance which is tasteless to some persons and extremely bitter to others. Ability to taste is inherited as a single gene difference, nontasting being recessive t/t .
- Punnett square (R. C. Punnett).** The conventional checkerboard with male gametes on one axis and female on the other. Zygotes are obtained by multiplying all male gametes by all female gametes.
- Pure line.** A line that has been rendered almost completely homozygous by self-pollination in plants or by sib-mating in animals.
- Purine.** A nitrogen base, one of the components of DNA and RNA. Two purines found in both DNA and RNA are adenine and guanine.
- Pyrimidine.** A nitrogen base, one of the components of DNA and RNA. Two pyrimidines found in DNA are cytosine and thymine; those in RNA are cytosine and uracil.
- q .** Symbol for the frequency of a mutant allele in a population: $p + q = 1$, $q = 1 - p$.
- Quantitative inheritance.** Inheritance determined by several or many genes, so that no discrete classes occur in segregating generation; blending inheritance.
- Quartet.** The four cells (before separation) arising from a spore mother cell. In higher plants they become pollen grains when mature.
- R_1, R_2, R_3 , etc.** The first, second, third, etc., generations following any type of radiation to induce mutations.
- Random mating.** Mating in which phenotypes cause no selection in choosing mates.
- Recessive.** A term coined by Mendel to describe characters which recede completely in the F_1 . Action of the recessive allele is suppressed by the dominant (*see* Dominance).
- Reciprocal crosses.** Hybrids made by using the mutant type as female in one case and as male in the other.
- Recombination.** A new combination of linked genes other than the parental types, e.g., in an F_1 of AB/ab genotype. Both Ab and aB gametes would represent recombinations.

- Recon.** A subdivision of the gene representing the smallest unit that is interchangeable, but not divisible by recombination.
- Red-green color-blindness.** The inability to distinguish red and green colors. One type is determined in man by a sex-linked gene located in nonhomologous part of the X chromosome.
- Reduced number of chromosomes.** The gametic or haploid number of chromosomes (*see* Haploid). Some lower organisms have reduced number in somatic cells, e.g., *Neurospora*.
- Reduction division.** A special type of cell division in which the chromosomes pair before dividing, then divide to give gametes with just half the somatic number of chromosomes. One of two divisions at meiosis is reductional.
- Repulsion.** Linkage of a mutant allele with one of the wild type, e.g., in cross of $A/A\ b/b \times a/a\ B/B$, F_1 is Ab/aB and linked genes in gametes are Ab and aB (*see* Trans-heterozygote). The term is a misnomer, since two mutant types or two wild type alleles do not repel each other. Rather, they tend to come out of a cross in the same association in which they enter.
- Reverse mutations.** Mutations from a mutant type to the normal or wild type allele. Less frequent than the mutation from the wild type to the mutant.
- Ribonucleic acid. RNA.** A nucleic acid found mainly in the cytoplasm and in some viruses, where it constitutes the principal hereditary material. Thought to be material for translating the genetic information of DNA into action.
- Roan cattle (Shorthorn breed).** Hybrids between red and white types. No dominance is involved. The F_1 is red and white, distinguishable from both parents.
- Roan horses.** Horses having an interspersal of white hairs through the normal-colored coat. This is due to a dominant gene.
- Roentgen (r).** The international unit of the quantity of X rays or gamma rays. One r produces 1.6×10^{12} ion pairs. Named for William Roentgen, the discoverer of X rays.
- Seed.** The mature ovule containing a dormant plant embryo.
- Seed parent.** The female (pistillate) parent of plant hybrids.
- Segregation.** The separation of alleles during meiosis, giving rise to a separation of the F_2 progeny into distinct types.
- Selective mating.** Nonrandom mating. Phenotypic differences influence the selection of a mate.
- Self-fertilization.** Fertilization following application of plant's own pollen.
- Self-pollination.** Pollinating a plant with its own pollen; selfing.
- Self-sterility.** Incapability of producing seed when self-pollinated. Several alleles, S_1, S_2, S_3 , etc., are responsible for this phenomenon.
- Semilethal.** Partially, but not completely, lethal. Semilethality can be either gametic or zygotic.
- Semisterile maize.** Corn plants of which the ears are only about 50% filled with kernels. The pollen shows about 50% empty and nonviable grains. Translocation is a common cause.
- Semisterility.** The condition of zygotes that are partially (about 50%) fertile. Translocations produce zygotes that are semisterile.
- Sex chromosome.** A chromosome usually designated X or Y, particularly concerned with sex determination; e.g., in *Drosophila* and mammals including man, the female is XX, the male is XY.
- Sex linkage.** An unusual type of inheritance caused by genes located in the X chromosomes. All genes in chromosome 1 in *Drosophila* are sex-linked. The Y chromosome is practically devoid of genes.
- Sex-promoting alleles (Habrobracon).** Certain alleles xa, xb, xc , etc. concerned with sex determination. Females are always heterozygous, males homozygous or hemizygous.

- Sexual reproduction.** Reproduction by the production of male and female gametes, followed by fertilization.
- Sib mating.** The mating of *sisters* with *brothers*; hence the name.
- Sickle cell anemia.** A condition produced by an abnormal hemoglobin molecule in the homozygous condition, h^s/h^s . Red blood cells become sickle-shaped under reduced oxygen tension.
- Sickle cell trait.** Trait shown by individuals characterized as heterozygote for the sickle cell gene h^a/h^s . All of red blood cells will become sickle-shaped if oxygen is reduced sufficiently. More reduction is tolerated than by an h^s/h^s individual.
- Silkless maize.** A male type producing pollen but no silks, due to a recessive gene sk/sk .
- Single cross.** The hybrid of two pure lines (primarily in corn breeding).
- Somatic cell.** A cell in the body of the organism, with $2n$ chromosomes. The contrasting type is the germ cell, with n chromosomes.
- Somatic mutation.** A mutation in a somatic cell.
- Soybean segregation.** A chlorophyll mutant with no dominance which produces one lethal golden seedling, g/g , two light green G/g , and one normal green G/G .
- Sperm 1. (Spermatozoon, pl. spermatozoa).** The mature male germ cell of an animal. 2. The male gamete produced in the pollen grain or pollen tube in plants.
- Spermatid.** One of the four cells which arise by division of the secondary spermatocyte; it becomes a mature spermatozoon following a metamorphosis known as spermiogenesis.
- Spermatocyte.** Sperm mother cell ($2n$). A diploid cell that undergoes meiosis in spermatogenesis. At second division four haploid spermatids are formed. These become the spermatozoa.
- Spermatogenesis.** Formation of mature sperm in animals. *S.* involves two meiotic divisions of spermatocyte, one a reductional division.
- Spermatogonium (pl. spermatogonia).** A primordial male germ cell that gives rise to the primary spermatocyte.
- Spontaneous mutation.** A mutation that arises without the use of any known mutagen, whose cause is unknown.
- Sporocyte.** The spore mother cell of a plant. Microsporocytes produce pollen, megasporocytes produce the embryo sac following meiosis.
- Sporogenesis.** The formation of microspores (pollen) and megaspores (embryo sac) in plants.
- Sporophytic.** Of the spore-bearing portion of a plant—the main part of higher plants—normally diploid ($2n$).
- Sport.** A mutation.
- Staminate.** Producing stamens only (no pistils); of a male plant.
- Stepwise synthesis.** The production of metabolic products in discrete steps, gene-controlled enzymes initiating the reactions.
- Sterility.** Inability to produce offspring.
- Stigma.** The upper end of the pistil that receives the pollen.
- Streptomycin dependent *E. coli*.** A strain of *E. coli* that must have small amount of streptomycin (an antibiotic) to grow.
- Style.** The elongated portion of the pistil of a flower between the ovary and the stigma. The pollen tube grows down the style before fertilization.
- Subvital.** Characterized by a reduction in vitality; said of a gamete or zygote. The effect is less than a semilethal.
- Super female.** An abnormal type, almost completely sterile, in *Drosophila* with an X chromosome/autosome ratio greater than 1, e.g., $3X/2A = 1.5$.
- Super male.** An abnormal type, almost completely sterile, in *Drosophila* with the X chromosome/autosome ratio less than 0.5, e.g., $1X/3A = 0.33$.
- Suppressor gene.** One which suppresses the action of another gene or other genes (*see* Inhibitor gene).

Survival of fittest. The mechanism of natural selection. By "fittest" is meant "able to leave most progeny."

Synapsis. The conjugation or pairing of homologous chromosomes at meiosis.

Syndrome. A group of symptoms that occur together and characterize a disease.

Telocentric. Characterized by a centromere at one end of the chromosome.

Telophase. The last stage in cell division, in which the chromosomes are assembled at each end of the cell.

Temperate phage. In viral genetics, a virus that invades, but does not kill, bacterial cells (*see* Virulent phage).

Temperate-sensitive Daphnia. A mutant that dies at normal temperature, but is adapted to much higher temperatures.

Terminalization. The movement of a chiasma away from the centromere and towards the end of a tetrad.

Testcross. The cross of an F_1 by the homozygous recessive, useful in linkage studies. Contrast with backcross.

Tetrad. The group of four chromatids the results from pairing of homologous chromosomes and division of each chromosome into two chromatids.

Tetraploid. Having four genomes ($4n$) instead of two, as in a diploid.

Tetrasomic. Having two extra chromosomes of a given kind, making four of the kind in question ($2n + 2$).

Threshold. A term used in studying effects of radiation. Below a certain dose, if there is a threshold, there is no measurable effect. No threshold exists for most genetic effects.

Thymine. A nitrogen base, one of two pyrimidines found in DNA but not in RNA (*see* Uracil).

Ticking, in dogs. Flecks or "ticks" of color in white areas of the coat, caused by a dominant gene T .

Tortoise-shell cat. A female cat with black and yellow markings, and occasionally with white spots. The gene for black and yellow is sex-linked. The female cat is heterozygous. Same as calico cat.

Transduction. The transfer of genes for bacterial characters by means of a phage particle acting as a "messenger boy."

Transformation. The changing of the genotype of a microorganism by combining with a transforming principle supplied; this principle is DNA.

Transformer gene tra in *Drosophila*. A recessive gene which when homozygous tra/tra causes a female fly to be phenotypically male. Such "males" are sterile.

Trans-heterozygote. A heterozygote of two linked genes with a mutant and wild type allele linked, $a+/+b$. This is a more appropriate term than "repulsion phase" of linkage, since like alleles do not repel.

Trans-position pseudoallele. Same as trans-heterozygote. Pseudoalleles are very closely linked.

Translocation. The exchange of parts of two nonhomologous chromosomes, following breakage, either spontaneous or induced.

Trihybrid. A hybrid involving three gene pairs such as $A/a B/b C/c$, and the offspring from such a hybrid.

Triplicate genes. Three genes, any one of which produces the same phenotypic effect, e.g., red seed coat color in wheat may be produced by R_1, R_2, R_3 .

Triploid. Having three genomes or sets of chromosomes ($3n$).

Trisomic. An organism with one extra chromosome, $2n + 1$, instead of the normal diploid ($2n$).

Trisomic inheritance. Abnormal inheritance of a gene located in the extra chromosome of a trisomic.

Turner's syndrome. An abnormality in human beings: individuals are phenotypi-

cally females, but have rudimentary sexual organs and mammary glands. Such individuals have but one X chromosome and no Y, with a total of 45 chromosomes instead of the normal 46.

Univalent. An unpaired chromosome at meiosis; in contrast to bivalents, which are paired.

Unstable gene. A gene which mutates frequently.

Uracil. A nitrogen base, one of two pyrimidines found in RNA, but not DNA (*see* Thymine).

Variation. Differences in typical characters, a feature of all living organisms of a species. Genetics is concerned with the inheritance of such differences.

Variegation. Diversity in characters in the same organism, e.g., alternating patches of green and white in leaves of some plants.

Velans (*Oenothera*). A gene complex characterized by genes for red striped buds, punctuate stems, narrow leaves, white nerves, and no red flecks on rosette leaves (*see* Gaudens).

Virescent seedling. A seedling that emerges white, or pale green, and turns green.

Virulent phage. A virus that kills the host bacterium. The contrasting type is temperate phage.

Virus. A parasitic particle in plants and animals, sometimes causing disease, incapable of reproduction outside of the host cell. A v. is too small to be resolved by the light microscope, but is readily photographed with the electron microscope. It is the smallest "organism" in which genetic recombination occurs. Some viruses, e.g., tobacco mosaic, are nucleoproteins and can be obtained in crystalline form.

✱

X₁, X₂, X₃, etc. The first, second, third, etc., generations following X irradiation.

Xanthophyll. A yellow-colored compound C₄₀H₅₆O₂ found in plants.

X chromosome. One of the sex chromosomes. A female is XX in *Drosophila* and in many mammals, including man. A male is XY. In poultry, the male is XX, the female is XO.

XO condition. Having an X chromosome but no Y chromosome, as the female in poultry and some other birds.

Y chromosome. One of the sex chromosomes found in males of many animals. Males are XY. Y chromosomes are mostly heterochromatic, with few genes.

Yellow fat in rabbits. An interesting character conditioned by a recessive gene *y/y*, causing the lack of an enzyme to break down the yellow pigment derived from xanthophylls.

Zygote. The result of the fusion of male and female gametes; the individual that develops from such a fusion, usually diploid with 2n chromosomes.

Zygotene (zygonema). A stage in prophase, when paired chromosomes first are visible as long, paired threads. Z. precedes pachytene, where threads are somewhat shortened and thickened.

Zygotic checkerboard. A Punnett square in which zygotes rather than gametes are used. Zygotes segregating for one gene (*A/a*) are placed on one axis, those of another (*B/b*) on the other axis, so that the proportions of all classes of a dihybrid segregation may be calculated directly.

Zygotic lethal. A lethal gene whose effect is in the embryo, larva, or adult, in contrast to the gametic lethal effecting a gamete.

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